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Deutsches Patent- und Markenamt

Der Präsident
Im Auftrag

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CORYNEBACTERIUM GLUTAMICUM GENES ENCODING NOVEL PROTEINS

Background of the Invention

5 Certain products and by-products of naturally-occurring metabolic processes in cells have utility in a wide array of industries, including the food, feed, cosmetics, and pharmaceutical industries. These molecules, collectively termed 'fine chemicals', include organic acids, both proteinogenic and non-proteinogenic amino acids, nucleotides and nucleosides, lipids and fatty acids, diols, carbohydrates, aromatic 10 compounds, vitamins and cofactors, and enzymes. Their production is most conveniently performed through the large-scale culture of bacteria developed to produce and secrete large quantities of one or more desired molecules. One particularly useful organism for this purpose is *Corynebacterium glutamicum*, a gram positive, nonpathogenic bacterium. Through strain selection, a number of mutant strains have 15 been developed which produce an array of desirable compounds. However, selection of strains improved for the production of a particular molecule is a time-consuming and difficult process.

Summary of the Invention

20 This invention provides novel nucleic acid molecules which may be used to identify or classify *Corynebacterium glutamicum* or related species of bacteria. *C. glutamicum* is a gram positive, aerobic bacterium which is commonly used in industry for the large-scale production of a variety of fine chemicals, and also for the degradation of hydrocarbons (such as in petroleum spills) and for the oxidation of terpenoids. The 25 nucleic acid molecules therefore can be used to identify microorganisms which can be used to produce fine chemicals, e.g., by fermentation processes. While *C. glutamicum* itself is nonpathogenic, it is related to other *Corynebacterium* species, such as *Corynebacterium diphtheriae* (the causative agent of diphtheria), which are important human pathogens. The ability to identify the presence of *Corynebacterium* species 30 therefore also can have significant clinical relevance, e.g., diagnostic applications. Further, these nucleic acid molecules may serve as reference points for the mapping of the *C. glutamicum* genome, or of genomes of related organisms.

These novel nucleic acid molecules encode proteins, referred to herein as marker and fine chemical production (MCP) proteins. These MCP proteins may be involved, 35 for example, in the direct or indirect production of one or more fine chemicals from *C. glutamicum*. The MCP proteins of the invention may also participate in the degradation of hydrocarbons or the oxidation of terpenoids. These proteins may also be utilized for

the identification of *Corynebacterium glutamicum* or organisms related to *C. glutamicum*: the presence of an MCP protein specific to *C. glutamicum* and related species in a mixture of proteins may indicate the presence of one of these bacteria in the sample. Further, these MCP proteins may have homologues in plants or animals which

5 are involved in a disease state or condition: these proteins thus may serve as useful pharmaceutical targets for drug screening and the development of therapeutic compounds.

Given the availability of cloning vectors for use in *Corynebacterium glutamicum*, such as those disclosed in Sinskey et al., U.S. Patent No. 4,649,119, and

10 techniques for genetic manipulation of *C. glutamicum* and the related *Brevibacterium* species (e.g., *lactofermentum*) (Yoshihama et al., *J. Bacteriol.* 162: 591-597 (1985); Katsumata et al., *J. Bacteriol.* 159: 306-311 (1984); and Santamaria et al., *J. Gen Microbiol.* 130: 2237-2246 (1984)), the nucleic acid molecules of the invention may be utilized in the genetic engineering of this organism to modulate the production of one or

15 more fine chemicals. This modulation may be due to a direct effect of manipulation of a gene of the invention, or it may be due to an indirect effect of such manipulation. For example, by modifying the activity of a protein involved in the biosynthesis or degradation of a fine chemical (i.e., through mutagenesis of the corresponding gene), one may directly modulate the ability of the cell to synthesize or to degrade this

20 compound, thereby modulating the yield and/or efficiency of production of the fine chemical. Similarly, by modulating the activity of a protein which regulates a fine chemical metabolic pathway, one may directly influence whether the production of the desired compound is up- or down-regulated, either of which will modulate the yield or efficiency of production of the fine chemical from the cell.

25 Indirect modulation of fine chemical production may also result by modifying the activity of a protein of the invention (i.e., by mutagenesis of the corresponding gene) such that the overall ability of the cell to grow and divide or to remain viable and productive is increased. The production of fine chemicals from *C. glutamicum* is generally accomplished by the large-scale fermentative culture of these microorganisms.

30 conditions which are frequently suboptimal for growth and cell division. By engineering a protein of the invention (e.g., a stress response protein, a cell wall protein, or proteins involved in the metabolism of compounds necessary for cell growth and division to occur, such as nucleotides and amino acids) such that it is better able to survive, grow, and multiply in such conditions, it may be possible to increase the

35 number and productivity of such engineered *C. glutamicum* cells in large-scale culture, which in turn should result in increased yields and/or efficiency of production of one or more desired fine chemicals. Further, the metabolic pathways of any cell are necessarily

interrelated and coregulated. By altering the activity or regulation of any one metabolic pathway in *C. glutamicum* (i.e., by altering the activity of one of the proteins of the invention which participates in such a pathway), it is possible to concomitantly alter the activity or regulation of other metabolic pathways in this microorganism, which may be 5 directly involved in the synthesis or degradation of a fine chemical.

The invention provides novel nucleic acid molecules which encode proteins, referred to herein as MCP proteins, which are capable of, for example, modulating the production or efficiency of production of one or more fine chemicals from *C. glutamicum*, or of serving as identifying markers for *C. glutamicum* or related 10 organisms. Nucleic acid molecules encoding an MCP protein are referred to herein as MCP nucleic acid molecules. In a preferred embodiment, the MCP protein is capable of modulating the production or efficiency of production of one or more fine chemicals from *C. glutamicum*, or of serving as identifying markers for *C. glutamicum* or related organisms. Examples of such proteins include those encoded by the genes set forth in 15 Table 1.

Accordingly, one aspect of the invention pertains to isolated nucleic acid molecules (e.g., cDNAs) comprising a nucleotide sequence encoding an MCP protein or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection or amplification of MCP-encoding nucleic acid 20 (e.g., DNA or mRNA). In particularly preferred embodiments, the isolated nucleic acid molecule comprises one of the nucleotide sequences set forth in Appendix A or the coding region or a complement thereof of one of these nucleotide sequences. In other particularly preferred embodiments, the isolated nucleic acid molecule of the invention comprises a nucleotide sequence which hybridizes to or is at least about 50%, preferably 25 at least about 60%, more preferably at least about 70%, 80% or 90%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence set forth in Appendix A, or a portion thereof. In other preferred embodiments, the isolated nucleic acid molecule encodes one of the amino acid sequences set forth in Appendix B. The preferred MCP proteins of the present invention 30 also preferably possess at least one of the MCP activities described herein.

In another embodiment, the isolated nucleic acid molecule encodes a protein or portion thereof wherein the protein or portion thereof includes an amino acid sequence which is sufficiently homologous to an amino acid sequence of Appendix B, e.g., sufficiently homologous to an amino acid sequence of Appendix B such that the protein 35 or portion thereof maintains an MCP activity. Preferably, the protein or portion thereof encoded by the nucleic acid molecule maintains the ability to modulate the production or efficiency of production of one or more fine chemicals from *C. glutamicum*, or of

serving as an identifying marker for *C. glutamicum* or related organisms. In one embodiment, the protein encoded by the nucleic acid molecule is at least about 50%, preferably at least about 60%, and more preferably at least about 70%, 80%, or 90% and most preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous to an amino acid sequence of Appendix B (e.g., an entire amino acid sequence selected from those sequences set forth in Appendix B). In another preferred embodiment, the protein is a full length *C. glutamicum* protein which is substantially homologous to an entire amino acid sequence of Appendix B (encoded by an open reading frame shown in Appendix A).

10 In another preferred embodiment, the isolated nucleic acid molecule is derived from *C. glutamicum* and encodes a protein (e.g., an MCP fusion protein) which includes a biologically active domain which is at least about 50% or more homologous to one of the amino acid sequences of Appendix B and is able to modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, to 15 degrade hydrocarbons, to oxidize terpenoids, to serve as a target for drug development, or to serve as an identifying marker for *C. glutamicum* or related organisms, and which also includes heterologous nucleic acid sequences encoding a heterologous polypeptide or regulatory regions.

17 In another embodiment, the isolated nucleic acid molecule is at least 15 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising a nucleotide sequence of Appendix A. Preferably, the isolated nucleic acid molecule corresponds to a naturally-occurring nucleic acid molecule. More preferably, the isolated nucleic acid encodes a naturally-occurring *C. glutamicum* MCP protein, or a biologically active portion thereof.

22 Another aspect of the invention pertains to vectors, e.g., recombinant expression vectors, containing the nucleic acid molecules of the invention, and host cells into which such vectors have been introduced. In one embodiment, such a host cell is used to produce an MCP protein by culturing the host cell in a suitable medium. The MCP protein can then be isolated from the medium or the host cell.

27 Yet another aspect of the invention pertains to a genetically altered microorganism in which an MCP gene has been introduced or altered. In one embodiment, the genome of the microorganism has been altered by introduction of a nucleic acid molecule of the invention encoding wild-type or mutated MCP sequence as a transgene. In another embodiment, an endogenous MCP gene within the genome of the microorganism has been altered, e.g., functionally disrupted, by homologous recombination with an altered MCP gene. In a preferred embodiment, the microorganism belongs to the genus *Corynebacterium* or *Brevibacterium*, with

Corynebacterium glutamicum being particularly preferred. In a preferred embodiment, the microorganism is also utilized for the production of a desired compound, such as an amino acid, with lysine being particularly preferred.

Still another aspect of the invention pertains to an isolated MCP protein or a portion, e.g., a biologically active portion, thereof. In a preferred embodiment, the isolated MCP protein or portion thereof is capable of modulating the production or efficiency of production of one or more fine chemicals from *C. glutamicum*, or of serving as an identifying marker for *C. glutamicum* or related organisms. In another preferred embodiment, the isolated MCP protein or portion thereof is sufficiently homologous to an amino acid sequence of Appendix B such that the protein or portion thereof maintains the ability to, for example, modulate the production or efficiency of production of one or more fine chemicals from *C. glutamicum*, or to serve as identifying markers for *C. glutamicum* or related organisms.

The invention also provides an isolated preparation of an MCP protein. In preferred embodiments, the MCP protein comprises an amino acid sequence of Appendix B. In another preferred embodiment, the invention pertains to an isolated full length protein which is substantially homologous to an entire amino acid sequence of Appendix B (encoded by an open reading frame set forth in Appendix A). In yet another embodiment, the protein is at least about 50%, preferably at least about 60%, 20 and more preferably at least about 70%, 80%, or 90%, and most preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous to an entire amino acid sequence of Appendix B. In other embodiments, the isolated MCP protein comprises an amino acid sequence which is at least about 50% or more homologous to one of the amino acid sequences of Appendix B and is able to modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, to degrade hydrocarbons, to oxidize terpenoids, to serve as a target for drug development, or to serve as an identifying marker for *C. glutamicum* or related organisms.

Alternatively, the isolated MCP protein can comprise an amino acid sequence which is encoded by a nucleotide sequence which hybridizes, e.g., hybridizes under stringent conditions, or is at least about 50%, preferably at least about 60%, more preferably at least about 70%, 80%, or 90%, and even more preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous, to a nucleotide sequence of Appendix B. It is also preferred that the preferred forms of MCP proteins also have one or more of the MCP bioactivities described herein.

35 The MCP polypeptide, or a biologically active portion thereof, can be operatively linked to a non-MCP polypeptide to form a fusion protein. In preferred embodiments, this fusion protein has an activity which differs from that of the MCP

protein alone. In other preferred embodiments, this fusion protein is capable of modulating the yield, production and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, or of serving as an identifying marker for *C. glutamicum* or related organisms. In particularly preferred embodiments, integration of this fusion

5 protein into a host cell modulates production of a desired compound from the cell.

Another aspect of the invention pertains to a method for producing a fine chemical. This method involves the culturing of a cell containing a vector directing the expression of an MCP nucleic acid molecule of the invention, such that a fine chemical is produced. In a preferred embodiment, this method further includes the step of

10 obtaining a cell containing such a vector, in which a cell is transfected with a vector directing the expression of an MCP nucleic acid. In another preferred embodiment, this method further includes the step of recovering the fine chemical from the culture. In a particularly preferred embodiment, the cell is from the genus *Corynebacterium* or

Brevibacterium, or is selected from those strains set forth in Table 3.

15 Another aspect of the invention pertains to methods for modulating production of a molecule from a microorganism. Such methods include contacting the cell with an agent which modulates MCP protein activity or MCP nucleic acid expression such that a cell associated activity is altered relative to this same activity in the absence of the agent. In a preferred embodiment, the cell is modulated for one or more *C. glutamicum*

20 MCP protein activities, such that the yield, production, and/or efficiency of production of a desired fine chemical by this microorganism is improved. The agent which modulates MCP protein activity can be an agent which stimulates MCP protein activity or MCP nucleic acid expression. Examples of agents which stimulate MCP protein activity or MCP nucleic acid expression include small molecules, active MCP proteins,

25 and nucleic acids encoding MCP proteins that have been introduced into the cell.

Examples of agents which inhibit MCP activity or expression include small molecules and antisense MCP nucleic acid molecules.

Another aspect of the invention pertains to methods for modulating yields, production, and/or efficiency of production of a desired compound from a cell, involving the introduction of a wild-type or mutant MCP gene into a cell, either maintained on a separate plasmid or integrated into the genome of the host cell. If integrated into the genome, such integration can be random, or it can take place by homologous recombination such that the native gene is replaced by the introduced copy, causing the production of the desired compound from the cell to be modulated. In a preferred embodiment, said yields are increased. In another preferred embodiment, said fine chemical is a fine chemical. In a particularly preferred embodiment, said fine chemical is an amino acid. In especially preferred embodiments, said amino acid is L-lysine.

Detailed Description of the Invention

The present invention provides MCP nucleic acid and protein molecules. These MCP nucleic acid molecules may be utilized in the identification of *Corynebacterium glutamicum* or related organisms, in the mapping of the *C. glutamicum* genome (or a 5 genome of a closely related organism), or in the identification of microorganisms which may be used to produce fine chemicals, e.g., by fermentation processes. The proteins encoded by these nucleic acids may be utilized in the direct or indirect modulation of the production or efficiency of production of one or more fine chemicals from *C. glutamicum*, as identifying markers for *C. glutamicum* or related organisms, in the 10 oxidation of terpenoids or the degradation of hydrocarbons, or as targets for the development of therapeutic pharmaceutical compounds. Aspects of the invention are further explicated below.

I. Fine Chemicals

15 The term 'fine chemical' is art-recognized and includes molecules produced by an organism which have applications in various industries, such as, but not limited to, the pharmaceutical, agriculture, and cosmetics industries. Such compounds include organic acids, such as tartaric acid, itaconic acid, and diaminopimelic acid, both proteinogenic and non-proteinogenic amino acids, purine and pyrimidine bases, 20 nucleosides, and nucleotides (as described e.g. in Kuninaka, A. (1996) Nucleotides and related compounds, p. 561-612, in Biotechnology vol. 6, Rehm et al., eds. VCH: Weinheim, and references contained therein), lipids, both saturated and unsaturated fatty acids (e.g., arachidonic acid), diols (e.g., propane diol, and butane diol), carbohydrates (e.g., hyaluronic acid and trehalose), aromatic compounds (e.g., aromatic amines, 25 vanillin, and indigo), vitamins and cofactors (as described in Ullmann's Encyclopedia of Industrial Chemistry, vol. A27, "Vitamins", p. 443-613 (1996) VCH: Weinheim and references therein; and Ong, A.S., Niki, E. & Packer, L. (1995) "Nutrition, Lipids, Health, and Disease" Proceedings of the UNESCO/Confederation of Scientific and Technological Associations in Malaysia, and the Society for Free Radical Research - 30 Asia, held Sept. 1-3, 1994 at Penang, Malaysia, AOCS Press, (1995)), enzymes, and all other chemicals described in Gutcho (1983) Chemicals by Fermentation, Noyes Data Corporation, ISBN: 0818805086 and references therein. The metabolism and uses of certain of these fine chemicals are further explicated below.

35 A. Amino Acid Metabolism and Uses

Amino acids comprise the basic structural units of all proteins, and as such are essential for normal cellular functioning in all organisms. The term "amino acid" is art-

recognized. The proteinogenic amino acids, of which there are 20 species, serve as structural units for proteins, in which they are linked by peptide bonds, while the nonproteinogenic amino acids (hundreds of which are known) are not normally found in proteins (see Ullmann's Encyclopedia of Industrial Chemistry, vol. A2, p. 57-97 VCH: Weinheim (1985)). Amino acids may be in the D- or L- optical configuration, though L-amino acids are generally the only type found in naturally-occurring proteins. Biosynthetic and degradative pathways of each of the 20 proteinogenic amino acids have been well characterized in both prokaryotic and eukaryotic cells (see, for example, Stryer, L. Biochemistry, 3rd edition, pages 578-590 (1988)). The 'essential' amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), so named because they are generally a nutritional requirement due to the complexity of their biosynthesis, are readily converted by simple biosynthetic pathways to the remaining 11 'nonessential' amino acids (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine). Higher animals do retain the ability to synthesize some of these amino acids, but the essential amino acids must be supplied from the diet in order for normal protein synthesis to occur.

Aside from their function in protein biosynthesis, these amino acids are interesting chemicals in their own right, and many have been found to have various applications in the food, feed, chemical, cosmetics, agriculture, and pharmaceutical industries. Lysine is an important amino acid in the nutrition not only of humans, but also of monogastric animals such as poultry and swine. Glutamate is most commonly used as a flavor additive (mono-sodium glutamate, MSG) and is widely used throughout the food industry, as are aspartate, phenylalanine, glycine, and cysteine. Glycine, L-methionine and tryptophan are all utilized in the pharmaceutical industry. Glutamine, valine, leucine, isoleucine, histidine, arginine, proline, serine and alanine are of use in both the pharmaceutical and cosmetics industries. Threonine, tryptophan, and D/L-methionine are common feed additives. (Leuchtenberger, W. (1996) Amino acids - technical production and use, p. 466-502 in Rehm et al. (eds.) Biotechnology vol. 6, chapter 14a, VCH: Weinheim). Additionally, these amino acids have been found to be useful as precursors for the synthesis of synthetic amino acids and proteins, such as N-acetylcysteine, S-carboxymethyl-L-cysteine, (S)-5-hydroxytryptophan, and others described in Ullmann's Encyclopedia of Industrial Chemistry, vol. A2, p. 57-97, VCH: Weinheim, 1985.

The biosynthesis of these natural amino acids in organisms capable of producing them, such as bacteria, has been well characterized (for review of bacterial amino acid biosynthesis and regulation thereof, see Umbarger, H.E. (1978) *Ann. Rev. Biochem.* 47: 533-606). Glutamate is synthesized by the reductive amination of α -

ketoglutarate, an intermediate in the citric acid cycle. Glutamine, proline, and arginine are each subsequently produced from glutamate. The biosynthesis of serine is a three-step process beginning with 3-phosphoglycerate (an intermediate in glycolysis), and resulting in this amino acid after oxidation, transamination, and hydrolysis steps. Both 5 cysteine and glycine are produced from serine; the former by the condensation of homocysteine with serine, and the latter by the transferal of the side-chain β -carbon atom to tetrahydrofolate, in a reaction catalyzed by serine transhydroxymethylase. Phenylalanine, and tyrosine are synthesized from the glycolytic and pentose phosphate pathway precursors erythrose 4-phosphate and phosphoenolpyruvate in a 9-step 10 biosynthetic pathway that differ only at the final two steps after synthesis of prephenate. Tryptophan is also produced from these two initial molecules, but its synthesis is an 11-step pathway. Tyrosine may also be synthesized from phenylalanine, in a reaction catalyzed by phenylalanine hydroxylase. Alanine, valine, and leucine are all 15 biosynthetic products of pyruvate, the final product of glycolysis. Aspartate is formed from oxaloacetate, an intermediate of the citric acid cycle. Asparagine, methionine, threonine, and lysine are each produced by the conversion of aspartate. Isoleucine is formed from threonine. A complex 9-step pathway results in the production of histidine from 5-phosphoribosyl-1-pyrophosphate, an activated sugar.

Amino acids in excess of the protein synthesis needs of the cell cannot be stored. 20 and are instead degraded to provide intermediates for the major metabolic pathways of the cell (for review see Stryer, L. Biochemistry 3rd ed. Ch. 21 "Amino Acid Degradation and the Urea Cycle" p. 495-516 (1988)). Although the cell is able to convert unwanted amino acids into useful metabolic intermediates, amino acid production is costly in terms of energy, precursor molecules, and the enzymes necessary to synthesize them. 25 Thus it is not surprising that amino acid biosynthesis is regulated by feedback inhibition, in which the presence of a particular amino acid serves to slow or entirely stop its own production (for overview of feedback mechanisms in amino acid biosynthetic pathways see Stryer, L. Biochemistry, 3rd ed. Ch. 24: "Biosynthesis of Amino Acids and Heme" p. 575-600 (1988)). Thus, the output of any particular amino acid is limited by the amount 30 of that amino acid present in the cell.

B. Vitamin, Cofactor, and Nutraceutical Metabolism and Uses

Vitamins, cofactors, and nutraceuticals comprise another group of molecules which the higher animals have lost the ability to synthesize and so must ingest, although 35 they are readily synthesized by other organisms such as bacteria. These molecules are either bioactive substances themselves, or are precursors of biologically active substances which may serve as electron carriers or intermediates in a variety of

metabolic pathways. Aside from their nutritive value, these compounds also have significant industrial value as coloring agents, antioxidants, and catalysts or other processing aids. (For an overview of the structure, activity, and industrial applications of these compounds, see, for example, Ullman's Encyclopedia of Industrial Chemistry, "Vitamins" vol. A27, p. 443-613. VCH: Weinheim, 1996.) The term "vitamin" is art-
5 recognized, and includes nutrients which are required by an organism for normal functioning, but which that organism cannot synthesize by itself. The group of vitamins may encompass cofactors and nutraceutical compounds. The language "cofactor" includes nonproteinaceous compounds required for a normal enzymatic activity to occur. Such compounds may be organic or inorganic: the cofactor molecules of the
10 invention are preferably organic. The term "nutraceutical" includes dietary supplements having health benefits in plants and animals, particularly humans. Examples of such molecules are vitamins, antioxidants, and also certain lipids (e.g., polyunsaturated fatty acids).

15 The biosynthesis of these molecules in organisms capable of producing them, such as bacteria, has been largely characterized (Ullman's Encyclopedia of Industrial Chemistry, "Vitamins" vol. A27, p. 443-613. VCH: Weinheim, 1996; Michal, G. (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley & Sons; Ong, A.S., Niki, E. & Packer, L. (1995) "Nutrition, Lipids, Health, and
20 Disease" Proceedings of the UNESCO/Confederation of Scientific and Technological Associations in Malaysia, and the Society for Free Radical Research - Asia, held Sept. 1-3, 1994 at Penang, Malaysia. AOCS Press: Champaign, IL X, 374 S).

25 Thiamin (vitamin B₁) is produced by the chemical coupling of pyrimidine and thiazole moieties. Riboflavin (vitamin B₂) is synthesized from guanosine-5'-triphosphate (GTP) and ribose-5'-phosphate. Riboflavin, in turn, is utilized for the synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The family of compounds collectively termed 'vitamin B₆' (e.g., pyridoxine, pyridoxamine, pyridoxal-5'-phosphate, and the commercially used pyridoxin hydrochloride) are all derivatives of the common structural unit, 5-hydroxy-6-methylpyridine. Pantothenate (pantothenic acid, (R)-(+)-N-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-β-alanine) can be produced either by chemical synthesis or by fermentation. The final steps in pantothenate biosynthesis consist of the ATP-driven condensation of β-alanine and pantoic acid. The enzymes responsible for the biosynthesis steps for the conversion to pantoic acid to β-alanine and for the condensation to pantothenic acid are known. The metabolically active form of pantothenate is Coenzyme A, for which the biosynthesis proceeds in 5 enzymatic steps. Pantothenate, pyridoxal-5'-phosphate, cysteine and ATP are the precursors of Coenzyme A. These enzymes not only catalyze the formation of
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panthothenate, but also the production of (R)-pantoic acid, (R)-pantolacton, (R)-panthenol (provitamin B₅), pantetheine (and its derivatives) and coenzyme A.

Biotin biosynthesis from the precursor molecule pimeloyl-CoA in

microorganisms has been studied in detail and several of the genes involved have been

5 identified. Many of the corresponding proteins have been found to also be involved in Fe-cluster synthesis and are members of the nifS class of proteins. Lipoic acid is derived from octanoic acid, and serves as a coenzyme in energy metabolism, where it becomes part of the pyruvate dehydrogenase complex and the α -ketoglutarate dehydrogenase complex. The folates are a group of substances which are all derivatives

10 of folic acid, which is turn is derived from L-glutamic acid, p-amino-benzoic acid and 6-methylpterin. The biosynthesis of folic acid and its derivatives, starting from the metabolism intermediates guanosine-5'-triphosphate (GTP), L-glutamic acid and p-amino-benzoic acid has been studied in detail in certain microorganisms.

Corrinoids (such as the cobalamines and particularly vitamin B₁₂) and

15 porphyrines belong to a group of chemicals characterized by a tetrapyrrole ring system. The biosynthesis of vitamin B₁₂ is sufficiently complex that it has not yet been completely characterized, but many of the enzymes and substrates involved are now known. Nicotinic acid (nicotinate), and nicotinamide are pyridine derivatives which are also termed 'niacin'. Niacin is the precursor of the important coenzymes NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) and their reduced forms.

The large-scale production of these compounds has largely relied on cell-free chemical syntheses, though some of these chemicals have also been produced by large-scale culture of microorganisms, such as riboflavin. Vitamin B₆, pantothenate, and

20 biotin. Only Vitamin B₁₂ is produced solely by fermentation, due to the complexity of its synthesis. *In vitro* methodologies require significant inputs of materials and time, often at great cost.

C. Purine, Pyrimidine, Nucleoside and Nucleotide Metabolism and Uses

30 Purine and pyrimidine metabolism genes and their corresponding proteins are important targets for the therapy of tumor diseases and viral infections. The language "purine" or "pyrimidine" includes the nitrogenous bases which are constituents of nucleic acids, co-enzymes, and nucleotides. The term "nucleotide" includes the basic structural units of nucleic acid molecules, which are comprised of a nitrogenous base, a

35 pentose sugar (in the case of RNA, the sugar is ribose; in the case of DNA, the sugar is D-deoxyribose), and phosphoric acid. The language "nucleoside" includes molecules which serve as precursors to nucleotides, but which are lacking the phosphoric acid

moiety that nucleotides possess. By inhibiting the biosynthesis of these molecules, or their mobilization to form nucleic acid molecules, it is possible to inhibit RNA and DNA synthesis: by inhibiting this activity in a fashion targeted to cancerous cells, the ability of tumor cells to divide and replicate may be inhibited. Additionally, there are 5 nucleotides which may serve as energy stores (e.g., ADP, ATP) or as coenzymes (i.e., FAD and NAD).

Several publications have described the use of these chemicals for these medical indications, by influencing purine and/or pyrimidine metabolism (e.g. Christopherson, R.I. and Lyons, S.D. (1990) "Potent inhibitors of *de novo* pyrimidine and purine 10 biosynthesis as chemotherapeutic agents." *Med Res Reviews* 10: 505-548). Studies of enzymes involved in purine and pyrimidine metabolism have been focused on the development of new drugs which can be used, for example, as immunosuppressants or anti-proliferants (Smith, J.L. (1995) "Enzymes in nucleotide synthesis." *Curr. Opin. Struct. Biol.* 5: 752-757; (1995) *Biochem Soc. Transact.* 23: 877-902). However, purine 15 and pyrimidine bases, nucleosides and nucleotides have other utilities: as intermediates in the biosynthesis of several fine chemicals (e.g., thiamine, S-adenosyl-methionine, folates, or riboflavin), as energy carriers for the cell (e.g., ATP or GTP), and for chemicals themselves, commonly used as flavor enhancers (e.g., IMP or GMP) or for several medicinal applications (see, for example, Kuninaka, A. (1996) *Nucleotides and 20 Related Compounds in Biotechnology* vol. 6, Rehm et al., eds. VCH: Weinheim, p. 561-612). Also, enzymes involved in purine, pyrimidine, nucleoside, or nucleotide metabolism are increasingly serving as targets against which chemicals for crop protection, including fungicides, herbicides and insecticides, are developed.

The metabolism of these compounds in bacteria has been characterized (for 25 reviews see, for example, Zalkin, H. and Dixon, J.E. (1992) "*de novo* purine nucleotide biosynthesis", in: *Progress in Nucleic Acid Research and Molecular Biology*, vol. 42, Academic Press, p. 259-287; and Michal, G. (1999) "Nucleotides and Nucleosides", Chapter 8 in: *Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology*, Wiley: New York). Purine metabolism has been the subject of intensive research, and is 30 essential to the normal functioning of the cell. Impaired purine metabolism in higher animals can cause severe disease, such as gout. Purine nucleotides are synthesized from ribose-5-phosphate, in a series of steps through the intermediate compound inosine-5'-phosphate (IMP), resulting in the production of guanosine-5'-monophosphate (GMP) or adenine-5'-monophosphate (AMP), from which the triphosphate forms utilized as 35 nucleotides are readily formed. These compounds are also utilized as energy stores, so their degradation provides energy for many different biochemical processes in the cell. Pyrimidine biosynthesis proceeds by the formation of uridine-5'-monophosphate (UMP)

from ribose-5-phosphate. UMP, in turn, is converted to cytidine-5'-triphosphate (CTP). The deoxy- forms of all of these nucleotides are produced in a one step reduction reaction from the diphosphate ribose form of the nucleotide to the diphosphate deoxyribose form of the nucleotide. Upon phosphorylation, these molecules are able to 5 participate in DNA synthesis.

D. Trehalose Metabolism and Uses

Trehalose consists of two glucose molecules, bound in a, α -1,1 linkage. It is commonly used in the food industry as a sweetener, an additive for dried or frozen 10 foods, and in beverages. However, it also has applications in the pharmaceutical, cosmetics and biotechnology industries (see, for example, Nishimoto et al., (1998) U.S. Patent No. 5,759,610; Singer, M.A. and Lindquist, S. (1998) *Trends Biotech.* 16: 460-467; Paiva, C.L.A. and Panek, A.D. (1996) *Biochem. Ann. Rev.* 2: 293-314; and 15 Shiosaka, M. (1997) *J. Japan* 172: 97-102). Trehalose is produced by enzymes from many microorganisms and is naturally released into the surrounding medium, from which it can be collected using methods known in the art.

II. Elements and Methods of the Invention

The present invention is based, at least in part, on the discovery of novel 20 molecules, referred to herein as MCP nucleic acid molecules. These MCP nucleic acid molecules are useful not only for the identification of *C. glutamicum* or related bacterial species, but also as markers for the mapping of the *C. glutamicum* genome and in the identification of bacteria useful for the production of fine chemicals by, e.g., fermentative processes. The present invention is also based, at least in part, on the MCP 25 protein molecules encoded by these MCP nucleic acid molecules. These MCP proteins are capable of modulating the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, of serving as identifying markers for *C. glutamicum* or related organisms, of degrading hydrocarbons, and of serving as targets for the development of therapeutic pharmaceutical compounds. In one embodiment, the 30 MCP molecules of the invention directly or indirectly participate in one or more fine chemical metabolic pathways in *C. glutamicum*. In a preferred embodiment, the activity of the MCP molecules of the invention to indirectly or directly participate in such metabolic pathways has an impact on the production of a desired fine chemical by this microorganism. In a particularly preferred embodiment, the MCP molecules of the 35 invention are modulated in activity, such that the *C. glutamicum* metabolic pathways in which the MCP proteins of the invention participate are modulated in efficiency or

output, which either directly or indirectly modulates the production or efficiency of production of a desired fine chemical by *C. glutamicum*.

The language, "MCP protein" or "MCP polypeptide" includes proteins which are able to modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, to degrade hydrocarbons, to oxidize terpenoids, to serve as a target protein for drug screening or design, or to serve as identifying markers for *C. glutamicum* or related organisms. Examples of MCP proteins include those encoded by the MCP genes set forth in Table 1 and Appendix A. The terms "MCP gene" or "MCP nucleic acid sequence" include nucleic acid sequences encoding an MCP protein, which consist of a coding region and also corresponding untranslated 5' and 3' sequence regions. Examples of MCP genes include those set forth in Table 1. The terms "production" or "productivity" are art-recognized and include the concentration of the fermentation product (for example, the desired fine chemical) formed within a given time and a given fermentation volume (e.g., kg product per hour per liter). The term "efficiency of production" includes the time required for a particular level of production to be achieved (for example, how long it takes for the cell to attain a particular rate of output of a fine chemical). The term "yield" or "product/carbon yield" is art-recognized and includes the efficiency of the conversion of the carbon source into the product (i.e., fine chemical). This is generally written as, for example, kg product per kg carbon source. By increasing the yield or production of the compound, the quantity of recovered molecules, or of useful recovered molecules of that compound in a given amount of culture over a given amount of time is increased. The terms "biosynthesis" or a "biosynthetic pathway" are art-recognized and include the synthesis of a compound, preferably an organic compound, by a cell from intermediate compounds in what may be a multistep and highly regulated process. The terms "degradation" or a "degradation pathway" are art-recognized and include the breakdown of a compound, preferably an organic compound, by a cell to degradation products (generally speaking, smaller or less complex molecules) in what may be a multistep and highly regulated process. The language "metabolism" is art-recognized and includes the totality of the biochemical reactions that take place in an organism. The metabolism of a particular compound, then, (e.g., the metabolism of an amino acid such as glycine) comprises the overall biosynthetic, modification, and degradation pathways in the cell related to this compound.

In another embodiment, the MCP molecules of the invention are capable of modulating the production of a desired molecule, such as a fine chemical, in a microorganism such as *C. glutamicum*, either directly or indirectly. Using recombinant genetic techniques, one or more of the MCP proteins of the invention may be

manipulated such that its function is modulated. Such modulation of function may result in the modulation of the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*.

For example, by modifying the activity of a protein involved in the biosynthesis or degradation of a fine chemical (i.e., through mutagenesis of the corresponding gene), one may directly modulate the ability of the cell to synthesize or to degrade this compound, thereby modulating the yield and/or efficiency of production of the fine chemical. Similarly, by modulating the activity of a protein which regulates a fine chemical metabolic pathway, one may directly influence whether the production of the desired compound is up- or down-regulated, either of which will modulate the yield or efficiency of production of the fine chemical from the cell.

Indirect modulation of fine chemical production may also result by modifying the activity of a protein of the invention (i.e., by mutagenesis of the corresponding gene) such that the overall ability of the cell to grow and divide or to remain viable and productive is increased. The production of fine chemicals from *C. glutamicum* is generally accomplished by the large-scale fermentative culture of these microorganisms, conditions which are frequently suboptimal for growth and cell division. By engineering a protein of the invention (e.g., a stress response protein, a cell wall protein, or proteins involved in the metabolism of compounds necessary for cell growth and division to occur, such as nucleotides and amino acids) such that it is better able to survive, grow, and multiply in such conditions, it may be possible to increase the number and productivity of such engineered *C. glutamicum* cells in large-scale culture, which in turn should result in increased yields and/or efficiency of production of one or more desired fine chemicals. Further, the metabolic pathways of any cell are necessarily interrelated and coregulated. By altering the activity or regulation of any one metabolic pathway in *C. glutamicum* (i.e., by altering the activity of one of the proteins of the invention which participates in such a pathway), it is possible to concomitantly alter the activity or regulation of other metabolic pathways in this microorganism, which may be directly involved in the synthesis or degradation of a fine chemical.

The isolated nucleic acid sequences of the invention are contained within the genome of a *Corynebacterium glutamicum* strain available through the American Type Culture Collection, given designation ATCC 13032. The nucleotide sequences of the isolated *C. glutamicum* MCP nucleic acid molecules and the predicted amino acid sequences of the *C. glutamicum* MCP proteins are shown in Appendices A and B, respectively. Computational analyses were performed which classified and/or identified many of these nucleotide sequences as sequences having homology to *E. coli* or *Bacillus subtilis* genes.

The present invention also pertains to proteins which have an amino acid sequence which is substantially homologous to an amino acid sequence of Appendix B. As used herein, a protein which has an amino acid sequence which is substantially homologous to a selected amino acid sequence is least about 50% homologous to the 5 selected amino acid sequence, e.g., the entire selected amino acid sequence. A protein which has an amino acid sequence which is substantially homologous to a selected amino acid sequence can also be least about 50-60%, preferably at least about 60-70%, and more preferably at least about 70-80%, 80-90%, or 90-95%, and most preferably at least about 96%, 97%, 98%, 99% or more homologous to the selected amino acid 10 sequence.

The MCP protein or a biologically active portion or fragment thereof of the invention is able to modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, to degrade hydrocarbons, to oxidize terpenoids, to serve as a target for drug development, or to serve as an identifying 15 marker for *C. glutamicum* or related organisms.

Various aspects of the invention are described in further detail in the following subsections:

A. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that 20 encode MCP polypeptides or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes or primers for the identification or amplification of MCP-encoding nucleic acid (e.g., MCP DNA). These nucleic acid molecules may be used to identify *C. glutamicum* or related organisms, to map the genome of *C. glutamicum* or closely related bacteria, or to identify microorganisms 25 useful for the production of fine chemicals, e.g., by fermentative processes. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. This term also encompasses untranslated 30 sequence located at both the 3' and 5' ends of the coding region of the gene: at least about 100 nucleotides of sequence upstream from the 5' end of the coding region and at least about 20 nucleotides of sequence downstream from the 3' end of the coding region of the gene. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA. An "isolated" nucleic acid molecule is one which is 35 separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the

nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated MCP nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived (e.g. a *C. glutamicum* cell). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having a nucleotide sequence of Appendix A, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, a *C. glutamicum* MCP cDNA can be isolated from a *C. glutamicum* library using all or portion of one of the sequences of Appendix A as a hybridization probe and standard hybridization techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning. A Laboratory Manual*. 2nd. ed. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Moreover, a nucleic acid molecule encompassing all or a portion of one of the sequences of Appendix A can be isolated by the polymerase chain reaction using oligonucleotide primers designed based upon this sequence (e.g., a nucleic acid molecule encompassing all or a portion of one of the sequences of Appendix A can be isolated by the polymerase chain reaction using oligonucleotide primers designed based upon this same sequence of Appendix A). For example, mRNA can be isolated from normal endothelial cells (e.g., by the guanidinium-thiocyanate extraction procedure of Chirgwin et al. (1979) *Biochemistry* 18: 5294-5299) and cDNA can be prepared using reverse transcriptase (e.g., Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD; or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL) and random polynucleotide primers or oligonucleotide primers based upon one of the nucleotide sequences shown in Appendix A. Synthetic oligonucleotide primers for polymerase chain reaction amplification can be designed based upon one of the nucleotide sequences shown in Appendix A. A nucleic acid of the invention can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to an MCP nucleotide sequence can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises one of the nucleotide sequences shown in Appendix A. The sequences of Appendix A correspond to the *Corynebacterium glutamicum* MCP cDNAs of the invention. This cDNA comprises sequences encoding MCP proteins (i.e., "the coding region", indicated in each sequence in Appendix A), as well as 5' untranslated sequences and 3' untranslated sequences, also indicated in Appendix A. Alternatively, the nucleic acid molecule can comprise only the coding region of any of the sequences in Appendix A.

For the purposes of this application, it will be understood that each of the sequences set forth in Appendix A has an identifying RXA number having the designation "RXA" followed by 5 digits (i.e., RXA00003). Each of these sequences comprises up to three parts: a 5' upstream region, a coding region, and a downstream region. Each of these three regions is identified by the same RXA designation to eliminate confusion. The recitation "one of the sequences in Appendix A", then, refers to any of the sequences in Appendix A, which may be distinguished by their differing RXA designations. The coding region of each of these sequences is translated into a corresponding amino acid sequence, which is set forth in Appendix B. The sequences of Appendix B are identified by the same RXA designations as Appendix A, such that they can be readily correlated. For example, the amino acid sequence in Appendix B designated RXA00003 is a translation of the coding region of the nucleotide sequence of nucleic acid molecule RXA00003 in Appendix A.

In one embodiment, the nucleic acid molecules of the present invention are not intended to include those compiled in Table 2.

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of one of the nucleotide sequences shown in Appendix A, or a portion thereof. A nucleic acid molecule which is complementary to one of the nucleotide sequences shown in Appendix A is one which is sufficiently complementary to one of the nucleotide sequences shown in Appendix A such that it can hybridize to one of the nucleotide sequences shown in Appendix A, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleotide sequence which is at least about 50-60%, preferably at least about 60-70%, more preferably at least about 70-80%, 80-90%, or 90-95%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence shown in Appendix A, or a portion thereof. In an additional preferred embodiment, an isolated nucleic acid molecule of the invention comprises a

nucleotide sequence which hybridizes, e.g., hybridizes under stringent conditions, to one of the nucleotide sequences shown in Appendix A; or a portion thereof.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the coding region of one of the sequences in Appendix A, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of an MCP protein. The nucleotide sequences determined from the cloning of the MCP genes from *C. glutamicum* allows for the generation of probes and primers designed for use in identifying and/or cloning MCP homologues in other cell types and organisms, as well as MCP homologues from other *Corynebacteria* or related species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 40, 50 or 75 consecutive nucleotides of a sense strand of one of the sequences set forth in Appendix A, an anti-sense sequence of one of the sequences set forth in Appendix A, or naturally occurring mutants thereof. Primers based on a nucleotide sequence of Appendix A can be used in PCR reactions to clone MCP homologues. Probes based on the MCP nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, e.g. the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells which misexpress an MCP protein, such as by measuring a level of an MCP-encoding nucleic acid in a sample of cells, e.g., detecting MCP mRNA levels or determining whether a genomic MCP gene has been mutated or deleted.

In one embodiment, the nucleic acid molecule of the invention encodes a protein or portion thereof which includes an amino acid sequence which is sufficiently homologous to an amino acid sequence of Appendix B such that the protein or portion thereof maintains the ability to modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, to degrade hydrocarbons, to oxidize terpenoids, to serve as a target for drug development, or to serve as an identifying marker for *C. glutamicum* or related organisms. As used herein, the language "sufficiently homologous" refers to proteins or portions thereof which have amino acid sequences which include a minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain as an amino acid residue in one of the sequences of Appendix B) amino acid residues to an amino acid sequence of Appendix B such that the protein or portion thereof is able to modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C.*

glutamicum, to degrade hydrocarbons, to oxidize terpenoids, to serve as a target for drug development, or to serve as an identifying marker for *C. glutamicum* or related organisms. Examples of such activities are also described herein. Thus, "the function of an MCP protein" contributes to the overall regulation of one or more fine chemical 5 metabolic pathways, or to the degradation of a hydrocarbon, or to the oxidation of a terpenoid.

In another embodiment, the protein is at least about 50-60%, preferably at least about 60-70%, and more preferably at least about 70-80%, 80-90%, 90-95%, and most preferably at least about 96%, 97%, 98%, 99% or more homologous to an entire amino 10 acid sequence of Appendix B.

Portions of proteins encoded by the MCP nucleic acid molecules of the invention are preferably biologically active portions of one of the MCP proteins. As used herein, the term "biologically active portion of an MCP protein" is intended to include a portion, e.g., a domain/motif, of an MCP protein that modulates the yield, production, and/or 15 efficiency of production of one or more fine chemicals from *C. glutamicum*, that degrades hydrocarbons, that oxidizes terpenoids, that may serve as a target for drug development, or that may serve as an identifying marker for *C. glutamicum* or related organisms. To determine whether an MCP protein or a biologically active portion thereof can modulate the yield, production, and/or efficiency of production of one or 20 more fine chemicals from *C. glutamicum*, can degrade hydrocarbons, or can oxidize terpenoids, an assay of activity may be performed. Such assay methods are well known to those skilled in the art, as detailed in Example 8 of the Exemplification.

Additional nucleic acid fragments encoding biologically active portions of an MCP protein can be prepared by isolating a portion of one of the sequences in Appendix 25 B, expressing the encoded portion of the MCP protein or peptide (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the MCP protein or peptide.

The invention further encompasses nucleic acid molecules that differ from one of the nucleotide sequences shown in Appendix A (and portions thereof) due to degeneracy 30 of the genetic code and thus encode the same MCP protein as that encoded by the nucleotide sequences shown in Appendix A. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in Appendix B. In a still further embodiment, the nucleic acid molecule of the invention encodes a full length *C. glutamicum* protein which is 35 substantially homologous to an amino acid sequence of Appendix B (encoded by an open reading frame shown in Appendix A).

In addition to the *C. glutamicum* MCP nucleotide sequences shown in Appendix A, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of MCP proteins may exist within a population (e.g., the *C. glutamicum* population). Such genetic polymorphism in the 5 MCP gene may exist among individuals within a population due to natural variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding an MCP protein, preferably a *C. glutamicum* MCP protein. Such natural variations can typically result in 1-5% variance in the nucleotide sequence of the MCP gene. Any and all such nucleotide variations and 10 resulting amino acid polymorphisms in MCP that are the result of natural variation and that do not alter the functional activity of MCP proteins are intended to be within the scope of the invention.

Nucleic acid molecules corresponding to natural variants and non-*C. glutamicum* homologues of the *C. glutamicum* MCP cDNA of the invention can be isolated based on 15 their homology to the *C. glutamicum* MCP nucleic acid disclosed herein using the *C. glutamicum* cDNA, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15 nucleotides in length and hybridizes under stringent conditions to the nucleic acid 20 molecule comprising a nucleotide sequence of Appendix A. In other embodiments, the nucleic acid is at least 30, 50, 100, 250 or more nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are 25 such that sequences at least about 65%, more preferably at least about 70%, and even more preferably at least about 75% or more homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization 30 conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C. followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to a sequence of Appendix A corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an 35 RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein). In one embodiment, the nucleic acid encodes a natural *C. glutamicum* MCP protein.

In addition to naturally-occurring variants of the MCP sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into a nucleotide sequence of Appendix A, thereby leading to changes in the amino acid sequence of the encoded MCP protein, without altering the functional ability 5 of the MCP protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in a sequence of Appendix A. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of one of the MCP proteins (Appendix B) without altering the activity of said MCP protein, whereas an "essential" amino acid residue is required for 10 MCP protein activity. Other amino acid residues, however, (e.g., those that are not conserved or only semi-conserved in the domain having MCP activity) may not be essential for activity and thus are likely to be amenable to alteration without altering MCP activity.

Accordingly, another aspect of the invention pertains to nucleic acid molecules 15 encoding MCP proteins that contain changes in amino acid residues that are not essential for MCP activity. Such MCP proteins differ in amino acid sequence from a sequence contained in Appendix B yet retain at least one of the MCP activities described herein. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 20 50% homologous to an amino acid sequence of Appendix B and is able to modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, to degrade hydrocarbons, to oxidize terpenoids, to serve as a target for drug development, or to serve as an identifying marker for *C. glutamicum* or related organisms. Preferably, the protein encoded by the nucleic acid molecule is at least about 25 50-60% homologous to one of the sequences in Appendix B, more preferably at least about 60-70% homologous to one of the sequences in Appendix B, even more preferably at least about 70-80%, 80-90%, 90-95% homologous to one of the sequences in Appendix B, and most preferably at least about 96%, 97%, 98%, or 99% homologous to one of the sequences in Appendix B.

30 To determine the percent homology of two amino acid sequences (e.g., one of the sequences of Appendix B and a mutant form thereof) or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of one protein or nucleic acid for optimal alignment with the other protein or nucleic acid). The amino acid residues or nucleotides at corresponding amino acid 35 positions or nucleotide positions are then compared. When a position in one sequence (e.g., one of the sequences of Appendix B) is occupied by the same amino acid residue or nucleotide as the corresponding position in the other sequence (e.g., a mutant form of

the sequence selected from Appendix B), then the molecules are homologous at that position (i.e., as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity"). The percent homology between the two sequences is a function of the number of identical positions shared by the sequences

5 (i.e., % homology = # of identical positions/total # of positions x 100).

An isolated nucleic acid molecule encoding an MCP protein homologous to a protein sequence of Appendix B can be created by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of Appendix A such that one or more amino acid substitutions, additions or deletions are introduced into the 10 encoded protein. Mutations can be introduced into one of the sequences of Appendix A by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a 15 similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, 20 proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an MCP protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly 25 along all or part of an MCP coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for an MCP activity described herein to identify mutants that retain MCP activity. Following mutagenesis of one of the sequences of Appendix A, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using, for example, assays described herein (see Example 8 of 30 the Exemplification).

In addition to the nucleic acid molecules encoding MCP proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., 35 complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be

complementary to an entire MCP coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an MCP protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are

5 translated into amino acid residues (e.g., the entire coding region of SEQ ID RXA00003 comprises nucleotides 1 to 741). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding MCP. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred

10 to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding MCP disclosed herein (e.g., the sequences set forth in Appendix A), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of MCP mRNA, but

15 more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of MCP mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of MCP mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed

20 by chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic

25 acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil,

30 dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiacytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-

5 amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target

5 nucleic acid of interest, described further in the following subsection).

10 The antisense nucleic acid molecules of the invention are typically administered to a cell or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an MCP protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. The antisense molecule can be modified such that it specifically binds to a receptor or an antigen expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecule to a peptide or

15 an antibody which binds to a cell surface receptor or antigen. The antisense nucleic acid molecule can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a eubacterial, viral or eucaryotic promoter are preferred.

20 In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-O-

25 methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

30 In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave MCP mRNA transcripts to thereby inhibit translation of MCP mRNA. A ribozyme having specificity for an MCP-encoding nucleic acid can be designed based upon the nucleotide sequence of an MCP cDNA disclosed herein (i.e., RXA00003 in Appendix A). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an MCP-encoding mRNA.

See, e.g., Cech et al. U.S. Patent No. 4,987,071 and Cech et al. U.S. Patent No. 5,116,742. Alternatively, MCP mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

5 Alternatively, MCP gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of an MCP nucleotide sequence (e.g., an MCP promoter and/or enhancers) to form triple helical structures that prevent transcription of an MCP gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al. (1992) *Ann N.Y. Acad. Sci.* 660:27-10 36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

B. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an MCP protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of 15 autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to 20 which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, 25 30 such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of

interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, repressor binding sites, activator binding sites, enhancer regions and other expression control elements (e.g., terminators, other elements of mRNA secondary structure, or polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185. Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in 5 many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides. 10 including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., MCP proteins, mutant forms of MCP proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of MCP proteins in prokaryotic or eukaryotic cells. For example, MCP genes can be expressed in bacterial cells such as *C. glutamicum*, insect cells (using 15 baculovirus expression vectors), yeast and other fungal cells (see Romanos, M.A. et al. (1992) "Foreign gene expression in yeast: a review", *Yeast* 8: 423-488; van den Hondel, C.A.M.J.J. et al. (1991) "Heterologous gene expression in filamentous fungi" in: *More Gene Manipulations in Fungi*, J.W. Bennet & L.L. Lasure, eds., p. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J. & Punt, P.J. (1991) "Gene transfer 20 systems and vector development for filamentous fungi, in: *Applied Molecular Genetics of Fungi*, Peberdy, J.F. et al., eds., p. 1-28. Cambridge University Press: Cambridge). algae and multicellular plant cells (see Schmidt, R. and Willmitzer, L. (1988) High 25 efficiency *Agrobacterium tumefaciens* -mediated transformation of *Arabidopsis thaliana* leaf and cotyledon explants" *Plant Cell Rep.*: 583-586), or mammalian cells. 30 Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185. Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out with vectors 35 containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion

vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase.

Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRJT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein. In one embodiment, the coding sequence of the MCP protein is cloned into a pGEX expression vector to create a vector encoding a fusion protein comprising, from the N-terminus to the C-terminus, GST-thrombin cleavage site-X protein. The fusion protein can be purified by affinity chromatography using glutathione-agarose resin. Recombinant MCP protein unfused to GST can be recovered by cleavage of the fusion protein with thrombin.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann et al., (1988) *Gene* 69:301-315) and pET 11d (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gnl0-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gnl). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gnl gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the bacterium chosen for expression, such as *C. glutamicum* (Wada et al. (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the MCP protein expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYEpSec1 (Baldari, et al., (1987) *Embo J.* 6:229-234), pMFA (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and methods for the construction of vectors appropriate for use in other fungi, such as the filamentous fungi, include those detailed in: van den Hondel, C.A.M.J.J. & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi. in: *Applied Molecular Genetics of Fungi*. J.F. Peberdy, et al., eds., p. 1-28, Cambridge University Press: Cambridge.

10 Alternatively, the MCP proteins of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf9 cells) include the pAc series (Smith et al. (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

15 In another embodiment, the MCP proteins of the invention may be expressed in unicellular plant cells (such as algae) or in plant cells from higher plants (e.g., the spermatophytes, such as crop plants). Examples of plant expression vectors include those detailed in: Becker, D., Kemper, E., Schell, J. and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", *Plant Mol. Biol.* 20: 1195-1197; and Bevan, M.W. (1984) "Binary *Agrobacterium* vectors for plant transformation", *Nucl. Acid. Res.* 12: 8711-8721.

20 In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd. ed.. Cold Spring Harbor Laboratory. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

25 In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific: Pinkert et al.

(1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters 5 (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kcsse and Gruss (1990) 10 *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in 15 a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to MCP mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which 20 direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene 25 expression using antisense genes see Weintraub, H. et al. (1986) "Antisense RNA as a molecular tool for genetic analysis", *Reviews - Trends in Genetics*, Vol. 1(1).

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such 30 terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

35 A host cell can be any prokaryotic or eukaryotic cell. For example, an MCP protein can be expressed in bacterial cells such as *C. glutamicum*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other

suitable host cells are known to those skilled in the art. Microorganisms related to *Corynebacterium glutamicum* which may be conveniently used as host cells for the nucleic acid and protein molecules of the invention are set forth in Table 3.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via

- 5 conventional transformation or transfection techniques. As used herein, the terms "transformation", "transfection", "conjugation" and "transduction" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including using natural competence, chemical mediated transfer, calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated
- 10 transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (*Molecular Cloning. A Laboratory Manual*. 2nd. ed. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the

- 15 expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding an MCP protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by, for example, drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).
- 20

25 To create a homologous recombinant microorganism, a vector is prepared which contains at least a portion of an MCP gene into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the MCP gene.

Preferably, this MCP gene is a *Corynebacterium glutamicum* MCP gene, but it can be a homologue from a related bacterium or even from a mammalian, yeast, or insect source.

- 30 In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous MCP gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous MCP gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous MCP protein). In the homologous recombination vector, the altered portion of the MCP gene is flanked at its 5' and 3' ends by additional nucleic acid of the MCP
- 35

gene to allow for homologous recombination to occur between the exogenous MCP gene carried by the vector and an endogenous MCP gene in a microorganism. The additional flanking MCP nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, less than one kilobase of flanking

5 DNA (both at the 5' and 3' ends) is included in the vector (see e.g., Thomas, K.R., and Capecchi, M.R. (1987) *Cell* 51: 503 for a description of homologous recombination vectors). The vector is introduced into a microorganism (e.g., by electroporation) and cells in which the introduced MCP gene has homologously recombined with the endogenous MCP gene are selected, using art-known techniques.

10 In another embodiment, recombinant microorganisms can be produced which contain selected systems which allow for regulated expression of the introduced gene. For example, inclusion of an MCP gene on a vector placing it under control of the lac operon permits expression of the MCP gene in the presence of IPTG. Such regulatory systems are well known in the art.

15 A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) an MCP protein. Accordingly, the invention further provides methods for producing MCP proteins using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding an MCP protein has been introduced, or into which genome has been introduced a gene encoding a wild-type or altered MCP protein) in a suitable medium until MCP protein is produced. In another embodiment, the method further comprises isolating MCP proteins from the medium or the host cell.

25 *C. Isolated MCP Proteins*

Another aspect of the invention pertains to isolated MCP proteins, and biologically active portions thereof. An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material when produced by recombinant DNA techniques, or chemical precursors or other chemicals when 30 chemically synthesized. The language "substantially free of cellular material" includes preparations of MCP protein in which the protein is separated from cellular components of the cells in which it is naturally or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of MCP protein having less than about 30% (by dry weight) of non-MCP protein (also referred to herein 35 as a "contaminating protein"), more preferably less than about 20% of non-MCP protein, still more preferably less than about 10% of non-MCP protein, and most preferably less than about 5% non-MCP protein. When the MCP protein or biologically active portion

thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations of MCP protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of MCP protein having less than about 30% (by dry weight) of chemical precursors or non-MCP chemicals, more preferably less than 10 about 20% chemical precursors or non-MCP chemicals, still more preferably less than about 10% chemical precursors or non-MCP chemicals, and most preferably less than about 5% chemical precursors or non-MCP chemicals. In preferred embodiments, isolated proteins or biologically active portions thereof lack contaminating proteins from the same organism from which the MCP protein is derived. Typically, such proteins are produced by recombinant expression of, for example, a *C. glutamicum* MCP protein in a microorganism such as *C. glutamicum*.

An isolated MCP protein or a portion thereof of the invention is able to modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, to degrade hydrocarbons, to oxidize terpenoids, to serve as a target for drug development, or to serve as an identifying marker for *C. glutamicum* or related organisms. In preferred embodiments, the protein or portion thereof comprises an amino acid sequence which is sufficiently homologous to an amino acid sequence of Appendix B such that the protein or portion thereof maintains the ability to modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*, to degrade hydrocarbons, to oxidize terpenoids, to serve as a target for drug development, or to serve as an identifying marker for *C. glutamicum* or related organisms. The portion of the protein is preferably a biologically active portion as described herein. In another preferred embodiment, an MCP protein of the invention has an amino acid sequence shown in Appendix B. In yet another preferred embodiment, the MCP protein has an amino acid sequence which is encoded by a nucleotide sequence which hybridizes, e.g., hybridizes under stringent conditions, to a nucleotide sequence of Appendix A. In still another preferred embodiment, the MCP protein has an amino acid sequence which is encoded by a nucleotide sequence that is at least about 50-60%, preferably at least about 60-70%, more preferably at least about 70-80%, 80-90%, 90-95%, and even more preferably at least about 96%, 97%, 98%, 99% or more homologous to one of the amino acid sequences of Appendix B. The preferred MCP proteins of the present invention also preferably possess at least one of the MCP

activities described herein. For example, a preferred MCP protein of the present invention includes an amino acid sequence encoded by a nucleotide sequence which hybridizes, e.g., hybridizes under stringent conditions, to a nucleotide sequence of Appendix A. and which is able to modulate the yield, production, and/or efficiency of 5 production of one or more fine chemicals from *C. glutamicum*, to degrade hydrocarbons, to oxidize terpenoids, to serve as a target for drug development, or to serve as an identifying marker for *C. glutamicum* or related organisms.

In other embodiments, the MCP protein is substantially homologous to an amino acid sequence of Appendix B and retains the functional activity of the protein of one of 10 the sequences of Appendix B yet differs in amino acid sequence due to natural variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the MCP protein is a protein which comprises an amino acid sequence which is at least about 50-60%, preferably at least about 60-70%, and more preferably at least about 70-80, 80-90, 90-95%, and most preferably at least about 96%, 97%, 98%, 15 99% or more homologous to an entire amino acid sequence of Appendix B and which has at least one of the MCP activities described herein. In another embodiment, the invention pertains to a full length *C. glutamicum* protein which is substantially homologous to an entire amino acid sequence of Appendix B.

Biologically active portions of an MCP protein include peptides comprising 20 amino acid sequences derived from the amino acid sequence of an MCP protein, e.g., an amino acid sequence shown in Appendix B or the amino acid sequence of a protein homologous to an MCP protein, which include fewer amino acids than a full length MCP protein or the full length protein which is homologous to an MCP protein, and exhibit at least one activity of an MCP protein. Typically, biologically active portions 25 (peptides, e.g., peptides which are, for example, 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100 or more amino acids in length) comprise a domain or motif with at least one activity of an MCP protein. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the activities described herein. Preferably, the biologically 30 active portions of an MCP protein include one or more selected domains/motifs or portions thereof having biological activity.

MCP proteins are preferably produced by recombinant DNA techniques. For example, a nucleic acid molecule encoding the protein is cloned into an expression vector (as described above), the expression vector is introduced into a host cell (as 35 described above) and the MCP protein is expressed in the host cell. The MCP protein can then be isolated from the cells by an appropriate purification scheme using standard protein purification techniques. Alternative to recombinant expression, an MCP protein

polypeptide, or peptide can be synthesized chemically using standard peptide synthesis techniques. Moreover, native MCP protein can be isolated from cells (e.g., endothelial cells, bacterial cells, fungal cells or other cells), for example using an anti-MCP antibody, which can be produced by standard techniques utilizing an MCP protein or fragment thereof of this invention.

The invention also provides MCP chimeric or fusion proteins. As used herein, an MCP "chimeric protein" or "fusion protein" comprises an MCP polypeptide operatively linked to a non-MCP polypeptide. An "MCP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an MCP protein, whereas a "non-MCP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the MCP protein, e.g., a protein which is different from the MCP protein and which is derived from the same or a different organism. Within the fusion protein, the term "operatively linked" is intended to indicate that the MCP polypeptide and the non-MCP polypeptide are fused 10 in-frame to each other. The non-MCP polypeptide can be fused to the N-terminus or C-terminus of the MCP polypeptide. For example, in one embodiment the fusion protein is a GST-MCP fusion protein in which the MCP sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of 15 recombinant MCP proteins. In another embodiment, the fusion protein is an MCP protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells, bacterial host cells, fungal host cells), expression and/or 20 secretion of an MCP protein can be increased through use of a heterologous signal sequence.

Preferably, an MCP chimeric or fusion protein of the invention is produced by 25 standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid 30 undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene 35 fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). An MCP-

encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the MCP protein.

Homologues of the MCP protein can be generated by mutagenesis, e.g., discrete point mutation or truncation of the MCP protein. As used herein, the term "homologue" 5 refers to a variant form of the MCP protein which acts as an agonist or antagonist of the activity of the MCP protein. An agonist of the MCP protein can retain substantially the same, or a subset, of the biological activities of the MCP protein. An antagonist of the MCP protein can inhibit one or more of the activities of the naturally occurring form of the MCP protein, by, for example, competitively binding to a downstream or upstream 10 member of a biochemical pathway which includes the MCP protein.

In an alternative embodiment, homologues of the MCP protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the MCP protein for MCP protein agonist or antagonist activity. In one embodiment, a variegated library of MCP variants is generated by combinatorial mutagenesis at the nucleic acid 15 level and is encoded by a variegated gene library. A variegated library of MCP variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential MCP sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of MCP sequences therein. 20 There are a variety of methods which can be used to produce libraries of potential MCP homologues from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding 25 the desired set of potential MCP sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of the MCP protein coding can be used to 30 generate a variegated population of MCP fragments for screening and subsequent selection of homologues of an MCP protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an MCP coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to 35 form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression

vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the MCP protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of MCP homologues. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify MCP homologues (Arkin and Yourvan (1992) *PNAS* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

In another embodiment, cell based assays can be exploited to analyze a variegated MCP library, using methods well known in the art.

20 *D. Uses and Methods of the Invention*

The nucleic acid molecules, proteins, protein homologues, fusion proteins, primers, vectors, and host cells described herein can be used in one or more of the following methods: identification of *C. glutamicum* and related organisms; mapping of genomes of organisms related to *C. glutamicum*; identification and localization of *C. glutamicum* sequences of interest; evolutionary studies; determination of MCP protein regions required for function; modulation of an MCP protein activity; modulation of the activity of one or more metabolic pathways; and modulation of cellular production of a desired compound, such as a fine chemical.

30 The MCP nucleic acid molecules of the invention have a variety of uses. First, they may be used to identify an organism as being *Corynebacterium glutamicum* or a close relative thereof. Also, they may be used to identify the presence of *C. glutamicum* or a relative thereof in a mixed population of microorganisms. The invention provides the nucleic acid sequences of a number of *C. glutamicum* genes, and probes based thereon; by probing the extracted genomic DNA of a culture of a unique or mixed population of microorganisms under stringent conditions with a probe spanning a region of a *C. glutamicum* gene which is unique to this organism, one can ascertain whether this organism is present. Although *Corynebacterium glutamicum* itself is

nonpathogenic, it is related to pathogenic species, such as *Corynebacterium diphtheriae*. Detection of such organisms is of significant clinical relevance.

To detect the presence of *C. glutamicum* in a sample, techniques well known in the art may be employed. Specifically, the cells in the sample may optionally first be

5 cultured in a suitable liquid or on a suitable solid culture medium to increase the number of cells in the sample. These cells are lysed, and the total DNA content extracted and optionally purified to remove debris and protein material which may interfere with subsequent analysis. The polymerase chain reaction or a similar technique known in the art is performed (for general reference on methodologies commonly used for the

10 amplification of nucleic acid sequences, see Mullis et al., U.S. Patent No. 4,683,195. Mullis et al., U.S. Patent No. 4,965,188, and Innis, M.A., and Gelfand, D. H., (1989) PCR Protocols. A guide to Methods and Applications, Academic Press, p. 3-12, and (1988) Biotechnology 6:1197, and International Patent Application No. WO89/01050) in which primers specific to an MCP nucleic acid molecule of the invention are

15 incubated with the nucleic acid sample such that, if present in the sample, that particular MCP nucleic acid sequence will be amplified. The particular MCP nucleic acid to be amplified is selected based on its uniqueness to the *C. glutamicum* genome, or to the genomes of *C. glutamicum* and only a few closely related bacteria. The presence of the desired amplified product is thus indicative of the presence of *C. glutamicum*, or an

20 organism closely related to *C. glutamicum*.

Further, the nucleic acid and protein molecules of the invention may serve as markers for specific regions of the genome. It is possible, using techniques well known in the art, to ascertain the physical location on the *C. glutamicum* genome of the MCP nucleic acid molecules of the invention, which in turn provides markers on the genome

25 which can be used to aid in the placement of other nucleic acid molecules and genes on the genome map. Also, the nucleic acid molecules of the invention may be sufficiently homologous to the sequences of related bacterial species that these nucleic acid molecules may similarly permit the construction of a genomic map in such bacteria (e.g., *Brevibacterium lactofermentum*).

30 The nucleic acid molecules of the invention have utility not only in the mapping of the genome, but also for functional studies of *C. glutamicum* proteins. For example, to identify the region of the genome to which a particular *C. glutamicum* DNA-binding protein binds, the *C. glutamicum* genome could be digested, and the fragments incubated with the DNA-binding protein. Those which bind the protein may be additionally probed

35 with the nucleic acid molecules of the invention, preferably with readily detectable labels; binding of such a nucleic acid molecule to the genome fragment enables the localization of the fragment to the genome map of *C. glutamicum*, and, when performed

multiple times with different enzymes, facilitates a rapid determination of the nucleic acid sequence to which the protein binds.

The MCP nucleic acid molecules of the invention are also useful for evolutionary and protein structural studies. The metabolic processes in which the molecules of the invention participate are utilized by a wide variety of prokaryotic and eukaryotic cells; by comparing the sequences of the nucleic acid molecules of the present invention to those encoding similar enzymes from other organisms, the evolutionary relatedness of the organisms can be assessed. Similarly, such a comparison permits an assessment of which regions of the sequence are conserved and which are not, which may aid in determining those regions of the protein which are essential for the functioning of the enzyme. This type of determination is of value for protein engineering studies and may give an indication of what the protein can tolerate in terms of mutagenesis without losing function.

The MCP protein molecules of the invention may also be utilized as markers for the classification of an unknown bacterium as *C. glutamicum*, or for the identification of *C. glutamicum* or closely related bacteria in a sample. For example, using techniques well known in the art, cells in a sample may optionally be amplified (e.g., by culturing in an appropriate medium) to increase the sample size, and then may be lysed to release proteins contained therein. This sample may optionally be purified to remove debris and nucleic acid molecules which may interfere with subsequent analysis. Antibodies specific for a selected MCP protein of the invention may be incubated with the protein sample in a typical Western assay format (see, e.g., Ausubel et al., (1988) *Current Protocols in Molecular Biology*, Wiley: New York) in which the antibody will bind to its target protein if this protein is present in the sample. An MCP protein is selected for this type of assay if it is unique or nearly unique to *C. glutamicum* or *C. glutamicum* and bacteria very closely related to *C. glutamicum*. Proteins in the sample are then separated by gel electrophoresis, and transferred to a suitable matrix, such as nitrocellulose. An appropriate secondary antibody having a detectable label (e.g., chemiluminescent or colorimetric) is incubated with this matrix, followed by stringent washing. The presence or absence of the label is indicative of the presence or absence of the target protein in the sample. If the protein is present, then this is indicative of the presence of *C. glutamicum*. A similar process enables the classification of an unknown bacterium as *C. glutamicum*; if a panel of proteins specific to *C. glutamicum* are not detected in protein samples prepared from the unknown bacterium, then that bacterium is not likely to be *C. glutamicum*.

Genetic manipulation of the MCP nucleic acid molecules of the invention may result in the production of MCP proteins having functional differences from the wild-

type MCP proteins. These proteins may be improved in efficiency or activity, may be present in greater numbers in the cell than is usual, or may be decreased in efficiency or activity.

Such changes in activity may directly modulate the yield, production, and/or efficiency of production of one or more fine chemicals from *C. glutamicum*. For example, by modifying the activity of a protein involved in the biosynthesis or degradation of a fine chemical (i.e., through mutagenesis of the corresponding gene), one may directly modulate the ability of the cell to synthesize or to degrade this compound, thereby modulating the yield and/or efficiency of production of the fine chemical. Similarly, by modulating the activity of a protein which regulates a fine chemical metabolic pathway, one may directly influence whether the production of the desired compound is up- or down-regulated, either of which will modulate the yield or efficiency of production of the fine chemical from the cell.

Indirect modulation of fine chemical production may also result by modifying the activity of a protein of the invention (i.e., by mutagenesis of the corresponding gene) such that the overall ability of the cell to grow and divide or to remain viable and productive is increased. The production of fine chemicals from *C. glutamicum* is generally accomplished by the large-scale fermentative culture of these microorganisms, conditions which are frequently suboptimal for growth and cell division. By engineering a protein of the invention (e.g., a stress response protein, a cell wall protein, or proteins involved in the metabolism of compounds necessary for cell growth and division to occur, such as nucleotides and amino acids) such that it is better able to survive, grow, and multiply in such conditions, it may be possible to increase the number and productivity of such engineered *C. glutamicum* cells in large-scale culture, which in turn should result in increased yields and/or efficiency of production of one or more desired fine chemicals. Further, the metabolic pathways of any cell are necessarily interrelated and coregulated. By altering the activity or regulation of any one metabolic pathway in *C. glutamicum* (i.e., by altering the activity of one of the proteins of the invention which participates in such a pathway), it is possible to concomitantly alter the activity or regulation of other metabolic pathways in this microorganism, which may be directly involved in the synthesis or degradation of a fine chemical.

The aforementioned mutagenesis strategies for MCP proteins to result in increased yields of a fine chemical from *C. glutamicum* are not meant to be limiting; variations on these strategies will be readily apparent to one skilled in the art. Using such strategies, and incorporating the mechanisms disclosed herein, the nucleic acid and protein molecules of the invention may be utilized to generate *C. glutamicum* or related strains of bacteria expressing mutated MCP nucleic acid and protein molecules such that

the yield, production, and/or efficiency of production of a desired compound is improved. This desired compound may be any natural product of *C. glutamicum*, which includes the final products of biosynthesis pathways and intermediates of naturally-occurring metabolic pathways, as well as molecules which do not naturally occur in the 5 metabolism of *C. glutamicum*, but which are produced by a *C. glutamicum* strain of the invention.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patent applications, patents, and published patent applications cited throughout this application are hereby incorporated by reference.

5

Exemplification

Example 1: Preparation of total genomic DNA of *Corynebacterium glutamicum* ATCC 13032

10 A culture of *Corynebacterium glutamicum* (ATCC 13032) was grown overnight at 30°C with vigorous shaking in BHI medium (Difco). The cells were harvested by centrifugation. the supernatant was discarded and the cells were resuspended in 5 ml buffer-I (5% of the original volume of the culture — all indicated volumes have been calculated for 100 ml of culture volume). Composition of buffer-I: 140.34 g/l sucrose.

15 2.46 g/l MgSO₄ x 7H₂O, 10 ml/l KH₂PO₄ solution (100 g/l, adjusted to pH 6.7 with KOH), 50 ml/l M12 concentrate (10 g/l (NH₄)₂SO₄, 1 g/l NaCl, 2 g/l MgSO₄ x 7H₂O, 0.2 g/l CaCl₂, 0.5 g/l yeast extract (Difco), 10 ml/l trace-elements-mix (200 mg/l FeSO₄ x H₂O, 10 mg/l ZnSO₄ x 7 H₂O, 3 mg/l MnCl₂ x 4 H₂O, 30 mg/l H₃BO₃, 20 mg/l CoCl₂ x 6 H₂O, 1 mg/l NiCl₂ x 6 H₂O, 3 mg/l Na₂MoO₄ x 2 H₂O, 500 mg/l complexing agent 20 (EDTA or citric acid), 100 ml/l vitamins-mix (0.2 mg/l biotin, 0.2 mg/l folic acid, 20 mg/l p-amino benzoic acid, 20 mg/l riboflavin, 40 mg/l ca-panthothenate, 140 mg/l nicotinic acid, 40 mg/l pyridoxole hydrochloride, 200 mg/l myo-inositol). Lysozyme was added to the suspension to a final concentration of 2.5 mg/ml. After an approximately 4 h incubation at 37°C, the cell wall was degraded and the resulting protoplasts are harvested by centrifugation. The pellet was washed once with 5 ml buffer-I and once with 5 ml TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). The pellet was resuspended in 4 ml TE-buffer and 0.5 ml SDS solution (10%) and 0.5 ml NaCl solution (5 M) are added. After adding of proteinase K to a final concentration of 200 µg/ml, the suspension is incubated for ca. 18 h at 37°C. The DNA was purified by 25 extraction with phenol, phenol-chloroform-isoamylalcohol and chloroform-isoamylalcohol using standard procedures. Then, the DNA was precipitated by adding 1/50 volume of 3 M sodium acetate and 2 volumes of ethanol, followed by a 30 min incubation at -20°C and a 30 min centrifugation at 12,000 rpm in a high speed centrifuge using a SS34 rotor (Sorvall). The DNA was dissolved in 1 ml TE-buffer containing 30 20

µg/ml RNaseA and dialysed at 4°C against 1000 ml TE-buffer for at least 3 hours. During this time, the buffer was exchanged 3 times. To aliquots of 0.4 ml of the dialysed DNA solution, 0.4 ml of 2 M LiCl and 0.8 ml of ethanol are added. After a 30 min incubation at -20°C, the DNA was collected by centrifugation (13.000 rpm, Biofuge Fresco, Heraeus, Hanau, Germany). The DNA pellet was dissolved in TE-buffer. DNA prepared by this procedure could be used for all purposes, including southern blotting or construction of genomic libraries.

Example 2: Construction of genomic libraries in *Escherichia coli* of *Corynebacterium glutamicum* ATCC13032.

Starting from DNA prepared as described in Example 1, cosmid and plasmid libraries were constructed according to known and well established methods (see e.g., Sambrook, J. et al. (1989) "Molecular Cloning : A Laboratory Manual", Cold Spring Harbor Laboratory Press, or Ausubel, F.M. et al. (1994) "Current Protocols in Molecular Biology", John Wiley & Sons.)

Any plasmid or cosmid could be used. Of particular use were the plasmids pBR322 (Sutcliffe, J.G. (1979) Proc. Natl. Acad. Sci. USA. 75:3737-3741); pACYC177 (Change & Cohen (1978) J. Bacteriol. 134:1141-1156), plasmids of the pBS series (pBSSK+, pBSSK- and others; Stratagene, LaJolla, USA), or cosmids as SuperCos1 (Stratagene, LaJolla, USA) or Lorist6 (Gibson, T.J., Rosenthal A. and Waterson, R.H. (1987) Gene 53:283-286.

Example 3: DNA Sequencing and Computational Functional Analysis

Genomic libraries as described in Example 2 were used for DNA sequencing according to standard methods, in particular by the chain termination method using ABI377 sequencing machines (see e.g., Fleischman, R.D. et al. (1995) "Whole-genome Random Sequencing and Assembly of *Haemophilus influenzae* Rd.", Science, 269:496-512). Sequencing primers with the following nucleotide sequences were used: 5'-GGAAACAGTATGACCATG-3' or 5'-GTAAAACGACGGCCAGT-3'.

30 Example 4: *In vivo* Mutagenesis

In vivo mutagenesis of *Corynebacterium glutamicum* can be performed by passage of plasmid (or other vector) DNA through *E. coli* or other microorganisms (e.g. *Bacillus* spp. or yeasts such as *Saccharomyces cerevisiae*) which are impaired in their capabilities to maintain

the integrity of their genetic information. Typical mutator strains have mutations in the genes for the DNA repair system (e.g., mutHLS, mutD, mutT, etc.; for reference, see Rupp, W.D. (1996) DNA repair mechanisms, in: *Escherichia coli and Salmonella*, p. 2277-2294. ASM: Washington.) Such strains are well known to those skilled in the art. The use of such strains is 5 illustrated, for example, in Greener, A. and Callahan, M. (1994) *Strategies* 7: 32-34.

Example 5: DNA Transfer Between *Escherichia coli* and *Corynebacterium glutamicum*

Several *Corynebacterium* and *Brevibacterium* species contain endogenous 10 plasmids (as e.g., pHM1519 or pBL1) which replicate autonomously (for review see, e.g., Martin, J.F. et al. (1987) *Biotechnology*, 5:137-146). Shuttle vectors for *Escherichia coli* and *Corynebacterium glutamicum* can be readily constructed by using standard vectors for 15 *E. coli* (Sambrook, J. et al. (1989), "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press or Ausubel, F.M. et al. (1994) "Current Protocols in Molecular Biology", John Wiley & Sons) to which a origin or replication for and a suitable marker from *Corynebacterium glutamicum* is added. Such origins of replication are preferably taken from endogenous plasmids isolated from *Corynebacterium* and *Brevibacterium* species. Of particular use as transformation markers for these species are 20 genes for kanamycin resistance (such as those derived from the Tn5 or Tn903 transposons) or chloramphenicol (Winnacker, E.L. (1987) "From Genes to Clones — Introduction to Gene Technology, VCH, Weinheim). There are numerous examples in the literature of the construction of a wide variety of shuttle vectors which replicate in both *E. coli* and *C. glutamicum*, and which can be used for several purposes, including gene over-expression (for reference, see e.g., Yoshihama, M. et al. (1985) *J. Bacteriol.* 162:591-597, 25 Martin J.F. et al. (1987) *Biotechnology*, 5:137-146 and Eikmanns, B.J. et al. (1991) *Gene*, 102:93-98).

Using standard methods, it is possible to clone a gene of interest into one of the shuttle vectors described above and to introduce such a hybrid vectors into strains of *Corynebacterium glutamicum*. Transformation of *C. glutamicum* can be achieved by 30 protoplast transformation (Kastsumata, R. et al. (1984) *J. Bacteriol.* 159:306-311), electroporation (Liebl, E. et al. (1989) *FEMS Microbiol. Letters*, 53:399-303) and in cases where special vectors are used, also by conjugation (as described e.g. in Schäfer, A. et al. (1990) *J. Bacteriol.* 172:1663-1666). It is also possible to transfer the shuttle vectors for

C. glutamicum to *E. coli* by preparing plasmid DNA from *C. glutamicum* (using standard methods well-known in the art) and transforming it into *E. coli*. This transformation step can be performed using standard methods, but it is advantageous to use an *Mcr*-deficient *E. coli* strain, such as NM522 (Gough & Murray (1983) *J. Mol. Biol.* 166:1-19).

5

Example 6: Assessment of the Expression of the Mutant Protein

Observations of the activity of a mutated protein in a transformed host cell rely on the fact that the mutant protein is expressed in a similar fashion and in a similar quantity to that of the wild-type protein. A useful method to ascertain the level of transcription of the mutant gene (an indicator of the amount of mRNA available for translation to the gene product) is to perform a Northern blot (for reference see, for example, Ausubel et al. (1988) *Current Protocols in Molecular Biology*, Wiley: New York), in which a primer designed to bind to the gene of interest is labeled with a detectable tag (usually radioactive or chemiluminescent), such that when the total RNA of a culture of the organism is extracted, run on gel, transferred to a stable matrix and incubated with this probe, the binding and quantity of binding of the probe indicates the presence and also the quantity of mRNA for this gene. This information is evidence of the degree of transcription of the mutant gene. Total cellular RNA can be prepared from *Corynebacterium glutamicum* by several methods, all well-known in the art, such as that described in Bormann, E.R. et al. (1992) *Mol. Microbiol.* 6: 317-326.

To assess the presence or relative quantity of protein translated from this mRNA, standard techniques, such as a Western blot, may be employed (see, for example, Ausubel et al. (1988) *Current Protocols in Molecular Biology*, Wiley: New York). In this process, total cellular proteins are extracted, separated by gel electrophoresis, transferred to a matrix such as nitrocellulose, and incubated with a probe, such as an antibody, which specifically binds to the desired protein. This probe is generally tagged with a chemiluminescent or colorimetric label which may be readily detected. The presence and quantity of label observed indicates the presence and quantity of the desired mutant protein present in the cell.

30

Example 7: Growth of Genetically Modified *Corynebacterium glutamicum* — Media and Culture Conditions

Genetically modified *Corynebacteria* are cultured in synthetic or natural growth media. A number of different growth media for *Corynebacteria* are both well-known and readily available (Lieb *et al.* (1989) *Appl. Microbiol. Biotechnol.*, 32:205-210; von der Osten *et al.* (1998) *Biotechnology Letters*, 11:11-16; Patent DE 4,120,867; Liebl (1992) "The Genus *Corynebacterium*, in: *The Prokaryotes*, Volume II. Balows, A. *et al.*, eds. Springer-Verlag). These media consist of one or more carbon sources, nitrogen sources, inorganic salts, vitamins and trace elements. Preferred carbon sources are sugars, such as mono-, di-, or polysaccharides. For example, glucose, fructose, mannose, galactose, ribose, sorbose, ribulose, lactose, maltose, sucrose, raffinose, starch or cellulose serve as very good carbon sources. It is also possible to supply sugar to the media via complex compounds such as molasses or other by-products from sugar refinement. It can also be advantageous to supply mixtures of different carbon sources. Other possible carbon sources are alcohols and organic acids, such as methanol, ethanol, acetic acid or lactic acid. Nitrogen sources are usually organic or inorganic nitrogen compounds, or materials which contain these compounds. Exemplary nitrogen sources include ammonia gas or ammonia salts, such as NH₄Cl or (NH₄)₂SO₄, NH₄OH, nitrates, urea, amino acids or complex nitrogen sources like corn steep liquor, soy bean flour, soy bean protein, yeast extract, meat extract and others.

Inorganic salt compounds which may be included in the media include the chloride-, phosphorous- or sulfate- salts of calcium, magnesium, sodium, cobalt, molybdenum, potassium, manganese, zinc, copper and iron. Chelating compounds can be added to the medium to keep the metal ions in solution. Particularly useful chelating compounds include dihydroxyphenols, like catechol or protocatechuate, or organic acids, such as citric acid. It is typical for the media to also contain other growth factors, such as vitamins or growth promoters, examples of which include biotin, riboflavin, thiamin, folic acid, nicotinic acid, pantothenate and pyridoxin. Growth factors and salts frequently originate from complex media components such as yeast extract, molasses, corn steep liquor and others. The exact composition of the media compounds depends strongly on the immediate experiment and is individually decided for each specific case. Information about media optimization is available in the textbook "Applied Microbiol. Physiology, A Practical Approach (eds. P.M. Rhodes, P.F. Stanbury, IRL Press (1997) pp. 53-73, ISBN 0

19 963577 3). It is also possible to select growth media from commercial suppliers, like standard 1 (Merck) or BHI (grain heart infusion, DIFC) or others.

All medium components are sterilized, either by heat (20 minutes at 1.5 bar and 121 °C) or by sterile filtration. The components can either be sterilized together or, if necessary, separately. All media components can be present at the beginning of growth, or they can optionally be added continuously or batchwise.

Culture conditions are defined separately for each experiment. The temperature should be in a range between 15°C and 45°C. The temperature can be kept constant or can be altered during the experiment. The pH of the medium should be in the range of 5 to 10, 8.5, preferably around 7.0, and can be maintained by the addition of buffers to the media. An exemplary buffer for this purpose is a potassium phosphate buffer. Synthetic buffers such as MOPS, HEPES, ACES and others can alternatively or simultaneously be used. It is also possible to maintain a constant culture pH through the addition of NaOH or NH₄OH during growth. If complex medium components such as yeast extract are utilized, 15 the necessity for additional buffers may be reduced, due to the fact that many complex compounds have high buffer capacities. If a fermentor is utilized for culturing the micro-organisms, the pH can also be controlled using gaseous ammonia.

The incubation time is usually in a range from several hours to several days. This time is selected in order to permit the maximal amount of product to accumulate in the 20 broth. The disclosed growth experiments can be carried out in a variety of vessels, such as microtiter plates, glass tubes, glass flasks or glass or metal fermentors of different sizes. For screening a large number of clones, the microorganisms should be cultured in microtiter plates, glass tubes or shake flasks, either with or without baffles. Preferably 100 ml shake flasks are used, filled with 10% (by volume) of the required growth 25 medium. The flasks should be shaken on a rotary shaker (amplitude 25 mm) using a speed-range of 100 – 300 rpm. Evaporation losses can be diminished by the maintenance of a humid atmosphere; alternatively, a mathematical correction for evaporation losses should be performed.

If genetically modified clones are tested, an unmodified control clone or a control 30 clone containing the basic plasmid without any insert should also be tested. The medium is inoculated to an OD₆₀₀ of 0.5 – 1.5 using cells grown on agar plates, such as CM plates (10 g/l glucose, 2.5 g/l NaCl, 2 g/l urea, 10 g/l polypeptone, 5 g/l yeast extract, 5 g/l meat extract, 22 g/l NaCl, 2 g/l urea, 10 g/l polypeptone, 5 g/l yeast extract, 5 g/l meat extract,

22 g/l agar, pH 6.8 with 2M NaOH) that had been incubated at 30°C. Inoculation of the media is accomplished by either introduction of a saline suspension of *C. glutamicum* cells from CM plates or addition of a liquid preculture of this bacterium.

5 **Example 8 – *In vitro* Analysis of the Function of Mutant Proteins**

The determination of activities and kinetic parameters of enzymes is well established in the art. Experiments to determine the activity of any given altered enzyme must be tailored to the specific activity of the wild-type enzyme, which is well within the ability of one skilled in the art. Overviews about enzymes in general, as well as specific details concerning structure, kinetics, principles, methods, applications and examples for the determination of many enzyme activities may be found, for example, in the following references: Dixon, M.. and Webb, E.C.. (1979) Enzymes. Longmans: London; Fersht. (1985) Enzyme Structure and Mechanism. Freeman: New York; Walsh. (1979) Enzymatic Reaction Mechanisms. Freeman: San Francisco; Price. N.C.. Stevens, L. (1982) Fundamentals of Enzymology. Oxford Univ. Press: Oxford; Boyer, P.D., ed. (1983) The Enzymes, 3rd ed. Academic Press: New York; Bisswanger, H., (1994) Enzymkinetik. 2nd ed. VCH: Weinheim (ISBN 3527300325); Bergmeyer, H.U., Bergmeyer, J., Graßl, M., eds. (1983-1986) Methods of Enzymatic Analysis, 3rd ed., vol. I-XII, Verlag Chemie: Weinheim; and Ullmann's Encyclopedia of Industrial Chemistry (1987) vol. A9, "Enzymes". VCH: Weinheim. p. 352-363.

The activity of proteins which bind to DNA can be measured by several well-established methods, such as DNA band-shift assays (also called gel retardation assays). The effect of such proteins on the expression of other molecules can be measured using reporter gene assays (such as that described in Kolmar, H. et al. (1995) EMBO J. 14: 3895-3904 and references cited therein). Reporter gene test systems are well known and established for applications in both pro- and eukaryotic cells, using enzymes such as beta-galactosidase, green fluorescent protein, and several others.

The determination of activity of membrane-transport proteins can be performed according to techniques such as those described in Gennis, R.B. (1989) "Pores, Channels and Transporters", in Biomembranes, Molecular Structure and Function, Springer: Heidelberg. p. 85-137; 199-234; and 270-322.

Example 9: Analysis of Impact of Mutant Protein on the Production of the Desired Product

The effect of the genetic modification in *C. glutamicum* on production of a desired compound (such as an amino acid) can be assessed by growing the modified microorganism under suitable conditions (such as those described above) and analyzing

the medium and/or the cellular component for increased production of the desired product (i.e., an amino acid). Such analysis techniques are well known to one skilled in the art, and include spectroscopy, thin layer chromatography, staining methods of various kinds, enzymatic and microbiological methods, and analytical chromatography

5 such as high performance liquid chromatography (see, for example, Ullman, Encyclopedia of Industrial Chemistry, vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon, A. et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17; Rehm et al. (1993) Biotechnology, vol. 3, Chapter III: "Product recovery and purification", page

10 469-714, VCH: Weinheim; Belter, P.A. et al. (1988) Bioseparations: downstream processing for biotechnology, John Wiley and Sons; Kennedy, J.F. and Cabral, J.M.S. (1992) Recovery processes for biological materials, John Wiley and Sons; Shaeiwitz, J.A. and Henry, J.D. (1988) Biochemical separations, in: Ullmann's Encyclopedia of Industrial Chemistry, vol. B3, Chapter 11, page 1-27, VCH: Weinheim; and Dechow,

15 F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications.)

In addition to the measurement of the final product of fermentation, it is also possible to analyze other components of the metabolic pathways utilized for the production of the desired compound, such as intermediates and side-products, to

20 determine the overall efficiency of production of the compound. Analysis methods include measurements of nutrient levels in the medium (e.g., sugars, hydrocarbons, nitrogen sources, phosphate, and other ions), measurements of biomass composition and growth, analysis of the production of common metabolites of biosynthetic pathways, and measurement of gasses produced during fermentation. Standard methods for these

25 measurements are outlined in Applied Microbial Physiology, A Practical Approach, P.M. Rhodes and P.F. Stanbury, eds., IRL Press, p. 103-129: 131-163; and 165-192 (ISBN: 0199635773) and references cited therein.

Example 10: Purification of the Desired Product from *C. glutamicum* Culture

30 Recovery of the desired product from the *C. glutamicum* cells or supernatant of the above-described culture can be performed by various methods well known in the art. If the desired product is not secreted from the cells, the cells can be harvested from the culture by low-speed centrifugation, the cells can be lysed by standard techniques, such as mechanical force or sonication. The cellular debris is removed by centrifugation, and

35 the supernatant fraction containing the soluble proteins is retained for further purification of the desired compound. If the product is secreted from the *C. glutamicum*

cells, then the cells are removed from the culture by low-speed centrifugation, and the supernatant fraction is retained for further purification.

The supernatant fraction from either purification method is subjected to chromatography with a suitable resin, in which the desired molecule is either retained on a chromatography resin while many of the impurities in the sample are not, or where the impurities are retained by the resin while the sample is not. Such chromatography steps may be repeated as necessary, using the same or different chromatography resins. One skilled in the art would be well-versed in the selection of appropriate chromatography resins and in their most efficacious application for a particular molecule to be purified.

10 The purified product may be concentrated by filtration or ultrafiltration, and stored at a temperature at which the stability of the product is maximized.

There are a wide array of purification methods known to the art and the preceding method of purification is not meant to be limiting. Such purification techniques are described, for example, in Bailey, J.E. & Ollis, D.F. *Biochemical Engineering Fundamentals*, McGraw-Hill: New York (1986).

15 The identity and purity of the isolated compounds may be assessed by techniques standard in the art. These include high-performance liquid chromatography (HPLC), spectroscopic methods, staining methods, thin layer chromatography, NIRS, enzymatic assay, or microbiologically. Such analysis methods are reviewed in: Patek et al. (1994) *Appl. Environ. Microbiol.* 60: 133-140; Malakhova et al. (1996) *Biotehnologiya* 11: 27-32; and Schmidt et al. (1998) *Bioprocess Engineer.* 19: 67-70. Ulmann's Encyclopedia of Industrial Chemistry, (1996) vol. A27, VCH: Weinheim, p. 89-90, p. 521-540, p. 540-547, p. 559-566, 575-581 and p. 581-587; Michal, G. (1999) *Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology*, John Wiley and Sons; Fallon, A. et al. (1987) Applications of HPLC in Biochemistry in: *Laboratory Techniques in Biochemistry and Molecular Biology*, vol. 17.

Equivalents

Those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

TAZIE 1: GENES IN THE APPLICATION

Identification <u>Code</u>	Config.	Start	NT	Stop
RXA02213	GR00652	425	6	
RXA00911	GR00248	1259	1765	
RXA02032	GR00818	4160	4729	
RXA01707	GR00481	802	1629	
RXA00271	GR0041	3709	2720	
RXA02427	GR00707	3447	3061	
RXA00399	GR00807	830	1144	
RXA01188	GR00338	3742	2645	
RXA00150	GR00023	4085	4858	
RXA00318	GR00055	428	635	
RXA00318	GR00059	1	783	
RXA00555	GR00145	2555	1665	
RXA00657	GR00169	10882	9980	
RXA00930	GR00253	3841	3089	
RXA01198	GR00343	3422	3724	
RXA01888	GR00443	497	33	
RXA01651	GR00474	1553	2974	
RXA02425	GR00707	1	630	
RXA02573	GR00739	594	151	
RXA02685	GR00753	6497	6018	
RXA00889	GR00242	15241	15928	
RXA02006	GR00787	48	570	
RXA01636	GR00460	1548	2444	
RXA02271	GR00759	1373	636	
RXA00462	GR00118	3023	1644	
RXA01286	GR00387	14457	13423	
RXA01380	GR00403	2	2017	
RXA02329	GR00725	7943	8071	
RXA00927	GR00003	5142	5507	
RXA00117	GR00019	791	201	
RXA00247	GR00037	7097	6171	
RXA01815	GR00515	3294	4085	
RXA02138	GR00639	4409	4750	
RXA02107	GR00632	1536	1877	
RXA02180	GR00841	16813	16358	
RXA01986	GR00587	47	703	
RXA00411	GR00092	1685	1011	
RXA01982	GR00573	3001	1844	

Table 1, Page 2

Identification Code	Config.	NT Start	NT Stop
RXA02367	GR00687	2162	1554
RXA02884	GR10020	1695	2158
RXA02773	GR00782	6407	8027
RXA02840	GR00835	488	339
RXA01986	GR00585	88	624
RXA01195	GR00343	1411	1859
RXA00305	GR00051	1257	826
RXA02383	GR00692	608	6
RXA02735	GR00763	777	73
RXA00239	GR00036	5118	4534
RXA01091	GR00105	546	76
RXA02690	GR00754	14502	13405
RXA00867	GR00175	593	1177
RXA00356	GR00070	2851	104
RXA01628	GR00165	1284	677
RXA01719	GR00188	5283	6911
RXA01645	GR00466	10574	9989
RXA02070	GR00827	1733	2830
RXA00149	GR00066	J	1061
RXA02224	GR00868	1548	2633
RXA02848	GR00848	113	511
RXA00153	GR00023	7656	7231
RXA00417	GR00093	404	3100
RXA02443	GR001709	6818	7771
RXA00325	GR00057	6594	9218
RXA00874	GR00241	758	1846
RXA02403	GR001700	896	1860
RXA01271	GR00367	21467	21656
RXA01288	GR00357	19165	18526
RXA01646	GR00456	11513	10695
RXA01671	GR00466	854	1468
RXA00805	GR00215	2057	2938
RXA00008	GR00002	605	115
RXA01359	GR00193	6897	8038
RXA00881	GR00235	6	431
RXA01076	GR00300	4374	3355
RXA02244	GR00654	12058	13580
RXA01698	GR00475	799	203
RXA02545	GR00728	18749	18192
RXA02888	GR00754	12258	12924
RXA02689	GR00754	13405	13084
RXA02588	GR00741	13037	12354
RXA01387	GR00397	1518	1919
RXA01577	GR00438	8811	9185
RXA01985	GR00441	1226	800
RXA0192	GR00423	6133	5130
RXA01592	GR00447	J	1295

<u>Identification</u>	<u>Code</u>	<u>Config.</u>	<u>Start</u>	<u>NT</u>	<u>Stop</u>
RXA01597	GR00447	GR00035	6220	1401	1477
RXA01176	GR00035	GR00045	1980	1477	4460
RXA01748	GR00045	GR00059	1681	1169	3169
RXA02117	GR00059	GR00069	4166	8864	8864
RXA02141	GR00069	GR00079	8457	7435	7435
RXA02016	GR00069	GR00068	6902	6561	6561
RXA00473	GR00119	GR00019	5799	4	4
RXA00213	GR00016	GR00016	420	4	4
RXA00224	GR00018	GR00018	988	459	459
RXA00161	GR00024	GR00024	4893	5354	5354
RXA00183	GR00028	GR00028	7344	8195	8195
RXA00279	GR00043	GR00043	4001	2816	2816
RXA00474	GR00119	GR00119	6575	8152	8152
RXA02114	GR00065	GR00065	6179	5839	5839
RXA003560	GR00149	GR00149	256	492	492
RXA00587	GR00156	GR00156	13008	13490	13490
RXA02575	GR00719	GR00719	1907	3084	3084
RXA02824	GR00805	GR00805	531	4	4
RXA02849	GR00849	GR00849	2	283	2775
RXA01159	GR00328	GR00328	3089	2775	2775
RXA01023	GR00292	GR00292	1817	867	867
RXA01944	GR00558	GR00558	2	385	385
RXA01635	GR00454	GR00454	5575	8315	8315
RXA01636	GR00454	GR00454	6326	8898	8898
RXA01945	GR00559	GR00559	192	1633	1633
RXA01968	GR00587	GR00587	3295	2138	2138
RXA01452	GR00710	GR00710	5271	5092	5092
RXA02183	GR00641	GR00641	18663	19187	19187
RXA00614	GR00162	GR00162	1680	2594	2594
RXA01322	GR00385	GR00385	443	6	6
RXA01342	GR00089	GR00089	11296	12807	12807
RXA00054	GR00098	GR00098	8557	11469	11469
RXA00098	GR00014	GR00014	4746	5048	5048
RXA00097	GR00014	GR00014	5222	6382	6382
RXA00118	GR00019	GR00019	918	1172	1172
RXA00122	GR00019	GR00019	4220	5842	5842
RXA00134	GR00021	GR00021	1648	1079	1079
RXA00159	GR00024	GR00024	3868	2687	2687
RXA00185	GR00028	GR00028	9418	12045	12045
RXA00220	GR00032	GR00032	20666	20163	20163
RXA00248	GR00037	GR00037	7843	7121	7121
RXA00285	GR00046	GR00046	3	515	515
RXA00321	GR00057	GR00057	2411	597	597
RXA00322	GR00057	GR00057	3858	2555	2555
RXA00339	GR00059	GR00059	817	1533	1533
RXA00348	GR00086	GR00086	6653	6103	6103
RXA00422	GR00097	GR00097	423	6	6

<u>Identification Code</u>	<u>Config.</u>	<u>Start</u>	<u>NT</u>	<u>Stop</u>
RXA00428	GR00098	2657	2025	
RXA00491	GR00122	1057	638	
RXA00505	GR00126	1	252	
RXA00540	GR00139	2027	2289	
RXA00552	GR00145	2	718	
RXA00553	GR00145	742	1062	
RXA00571	GR00156	117	767	
RXA00574	GR00158	767	1645	
RXA00578	GR00156	4087	3365	
RXA00596	GR00156	12818	11937	
RXA00610	GR00161	1193	2056	
RXA00613	GR00162	1652	1200	
RXA00637	GR00167	2002	2754	
RXA00649	GR00169	2823	3278	
RXA00656	GR00175	390	4	
RXA00691	GR00181	2152	1223	
RXA00713	GR00188	71	1033	
RXA00716	GR00188	3002	3514	
RXA00722	GR00189	1015	512	
RXA00738	GR00201	78	365	
RXA00765	GR00204	3283	3969	
RXA00787	GR00204	5280	5993	
RXA00768	GR00204	5958	6398	
RXA00781	GR00206	2662	2395	
RXA00846	GR00220	391	5	
RXA00859	GR00234	4	636	
RXA00869	GR00239	1	792	
RXA00887	GR00242	13544	14266	
RXA00940	GR00257	129	524	
RXA00949	GR00259	5400	8047	
RXA00986	GR00280	60	401	
RXA00987	GR00280	875	411	
RXA01011	GR00288	2089	857	
RXA01017	GR00290	2175	1587	
RXA01021	GR00291	1759	2280	
RXA01074	GR00300	2811	2107	
RXA01078	GR00300	6043	6876	
RXA01088	GR00304	3083	1902	
RXA01129	GR00314	1461	3326	
RXA01198	GR00343	1889	2578	
RXA01197	GR00343	3333	2881	
RXA01207	GR00347	126	773	
RXA01237	GR00358	2751	2311	
RXA01246	GR00360	1824	2462	
RXA01249	GR00363	303	4	
RXA01251	GR00365	228	538	
RXA01282	GR00389	5444	4665	
RXA01294	GR00373	3537	2872	
RXA01348	GR00392	261	752	
RXA01357	GR00393	4357	4659	

<u>Identification</u>	<u>Code</u>	<u>NT</u>	<u>Stop</u>
RXA01382	GR00395	1397	
RXA01364	GR00398	4	
RXA01388	GR00397	1369	980
RXA01370	GR00399	1875	2225
RXA01372	GR00399	1	591
RXA01379	GR00402	928	6
RXA01398	GR00408	6475	6218
RXA01397	GR00408	6894	6475
RXA01409	GR00410	5298	4481
RXA01428	GR00417	5651	6268
RXA01439	GR00418	5949	6494
RXA01463	GR00421	2493	1330
RXA01488	GR00423	2179	1349
RXA01497	GR00424	262	1179
RXA01501	GR00424	8130	7843
RXA01505	GR00424	11318	11815
RXA01523	GR00424	27951	28901
RXA01595	GR00447	3328	4285
RXA01600	GR00447	10480	11128
RXA01622	GR00452	1908	2510
RXA01682	GR00462	1890	2432
RXA01709	GR00483	745	416
RXA01715	GR00485	1267	1962
RXA01738	GR00493	3971	4884
RXA01803	GR00509	5671	4712
RXA01804	GR00509	6117	5797
RXA01805	GR00509	6515	6186
RXA01844	GR00522	1950	1771
RXA01871	GR00534	2797	3759
RXA01875	GR00536	516	1313
RXA01877	GR00537	135	1199
RXA01879	GR00537	2117	2704
RXA01880	GR00537	2641	3048
RXA01898	GR00544	2	580
RXA01916	GR00549	1034	2044
RXA01911	GR00555	4913	6588
RXA01942	GR00557	1526	2927
RXA01892	GR00581	709	260
RXA02023	GR00613	3234	4001
RXA02057	GR00625	2972	3502
RXA02071	GR00628	458	6
RXA02104	GR00631	5327	4908
RXA02108	GR00632	2077	2511
RXA02117	GR00636	1056	1529
RXA02121	GR00636	6558	7928
RXA02124	GR00636	7856	8911
RXA02166	GR00640	13048	13224
RXA02177	GR00641	12683	13615
RXA02187	GR00641	21249	23447
RXA02211	GR00648	2537	2989

Identification Code	Config.	Start	NT	Stop
RXA02216	GR00651	2	307	
RXA02217	GR00851	969	306	
RXA02218	GR00651	1299	1565	
RXA02219	GR00651	1578	2983	
RXA02255	CR00654	22507	23442	
RXA02298	CR00662	10310	8652	
RXA02308	CR00684	939	511	
RXA02337	CR00672	2893	3816	
RXA02347	CR00677	509	189	
RXA02349	CR00678	394	5	
RXA02352	CR00681	2	556	
RXA02387	CR00694	683	6	
RXA02393	CR00697	168	449	
RXA02395	CR00698	2	733	
RXA02398	CR00698	1309	1031	
RXA02407	CR00701	1580	1885	
RXA02449	CR00702	1248	835	
RXA02450	CR00707	7498	7683	
RXA02459	CR00712	4341	5075	
RXA02472	CR00715	5435	5125	
RXA02484	CR00718	2317	1817	
RXA02486	CR00718	3441	4076	
RXA02496	CR00720	10025	9219	
RXA02514	CR00723	1	837	
RXA02518	CR00723	3464	3874	
RXA02521	CR00724	2924	4166	
RXA02525	CR00725	3113	3490	
RXA02540	CR00726	12438	12001	
RXA02601	CR00742	5258	7246	
RXA02617	CR00745	1404	1910	
RXA02639	CR00749	511	1344	
RXA02672	CR00753	12503	13400	
RXA02714	CR00758	14754	14526	
RXA02720	CR00759	631	6	
RXA02761	CR00764	6393	5920	
RXA02766	CR00770	986	594	
RXA02789	CR00777	5237	5782	
RXA02798	CR00778	1648	1100	
RXA02804	CR10015	1348	869	
RXA02801	CR10040	9518	10195	
RXA01504	CR00424	10710	11318	
RXA01508	CR00424	11815	12225	
RXA01617	CR00458	12422	11535	
RXA01798	CR00508	2	484	
RXA02132	CR00638	737	1375	
RXA02254	CR00654	21769	22449	
RXA02482	CR00710	914	105	
RXA02789	CR00780	182	454	
RXA00052	CR00008	7957	7247	

<u>Identification Code</u>	<u>Conig.</u>	NT Start	NT Stop
RXA00160	GR00028	2334	1795
RXA00163	GR00204	1384	2168
RXA00926	GR00253	466	104
RXA01273	GR00387	26475	25042
RXA02798	GR00776	2842	4286
RXA02847	GR00847	598	5
RXA02898	GR10040	1631	6
RXA02899	GR10040	2125	1646
RXA00025	GR00003	2211	3647
RXA00193	GR00014	204	2428
RXA00101	GR00114	10514	10107
RXA00108	GR00015	546	4
RXA00197	GR00030	1731	2741
RXA00297	GR00048	2861	3772
RXA00391	GR00049	1970	2506
RXA00316	GR00057	19461	19931
RXA00444	GR00063	6	584
RXA00416	GR00093	1	327
RXA00418	GR00094	1	1065
RXA00430	GR00098	3473	3083
RXA00447	GR00108	518	817
RXA00455	CR00113	2	619
RXA00485	GR00119	25230	23188
RXA00490	GR00121	2876	1774
RXA00506	GR00126	489	1829
RXA00515	GR00131	1	482
RXA00520	GR00132	599	796
RXA00602	GR00159	4907	4155
RXA00811	GR00161	3640	2165
RXA00688	GR00176	797	6
RXA00874	GR00177	755	6
RXA00731	CR00185	2613	142
RXA00830	GR00224	266	988
RXA00815	GR00228	3	692
RXA01058	CR00298	2184	3254
RXA01177	GR00289	2822	2438
RXA01071	CR00006	10018	8774
RXA0119	CR00310	1068	139
RXA01158	GR00328	2580	1639
RXA01177	GR00335	2121	4108
RXA01229	GR00355	2806	3498
RXA01331	GR00387	1608	1031
RXA01507	GR00424	12239	12861
RXA01623	GR00452	2514	3224
RXA01624	GR00452	3220	3564
RXA01669	GR00463	1002	271
RXA01673	GR00487	1807	73
RXA01685	GR00470	1488	910

Identification Code	Config.	NT Start	NT Stop
RXA01749	GR00495	4833	6249
RXA01806	GR00509	6595	7074
RXA02080	GR00828	11017	10211
RXA02172	GR00841	6919	6581
RXA02295	GR00862	6842	6063
RXA02297	GR00862	7502	8838
RXA02290	GR00895	1500	872
RXA02408	GR00102	832	5
RXA02488	GR00719	1	369
RXA02489	GR00719	173	996
RXA02495	GR00720	9002	6435
RXA02524	GR00725	2405	3084
RXA02544	GR00728	16715	16142
RXA02584	GR00741	8925	8575
RXA02585	GR00741	9917	8937
RXA02598	GR00742	2576	3166
RXA02600	GR00742	5027	3830
RXA02602	GR00742	7239	7742
RXA02604	GR00742	8800	10875
RXA02693	GR00755	1650	4
RXA02700	GR00757	3507	4742
RXA02701	GR00757	4838	6145
RXA00554	GR00169	7213	8478
RXA01125	GR00417	1701	2585
RXA02549	GR00728	1331	6
RXA02379	GR00740	4385	3818
RXA02580	GR00740	4982	4239
RXA00008	GR00216	277	5
RXA00008	GR00217	1029	352
RXA01318	GR00382	3618	2315
RXA01677	GR00467	5043	4300
RXA01658	GR00461	5	1489
RXA02597	GR00757	1	699
RXA02718	GR00758	19598	20245
RXA00003	GR00001	2279	3019
RXA00015	GR00002	5999	6307
RXA00018	GR00002	12979	14277
RXA00020	GR00002	17142	16363
RXA00021	GR00002	19766	20538
RXA00022	GR00002	20583	21297
RXA00028	GR00003	8058	6112
RXA00031	GR00003	10183	8982
RXA00036	GR00004	7204	8619
RXA00037	GR00004	9557	8685
RXA00039	GR00006	2099	1431
RXA00040	GR00006	2499	2095

Identification Code	NT	Stop	NT
		Start	Config.
RXA00047	CR00008	514	95
RXA00049	GR00008	2270	2956
RXA00058	GR00009	1463	714
RXA00058	GR00009	7394	6831
RXA00059	GR00009	8301	8020
RXA00060	GR00010	1658	1374
RXA00060	GR00010	4140	4412
RXA00067	GR00011	708	223
RXA00068	GR00011	1305	724
RXA00077	GR00012	4228	5589
RXA00079	GR00012	8599	6820
RXA00080	GR00012	7342	8923
RXA00082	GR00012	9019	8456
RXA00083	GR00013	771	1070
RXA00086	GR00013	2739	3092
RXA00087	GR00013	1704	2462
RXA00094	GR00014	1983	3456
RXA00094	GR00014	3163	3435
RXA00110	GR00016	364	912
RXA00114	GR00017	3420	3908
RXA00119	GR00019	1704	2462
RXA00120	GR00019	2798	3451
RXA00121	GR00018	3473	4183
RXA00127	GR00020	2871	2416
RXA00128	GR00020	4709	3006
RXA00140	GR00022	3841	3658
RXA00141	GR00022	4307	3846
RXA00142	GR00022	4776	4300
RXA00151	GR00023	4958	5552
RXA00154	GR00023	8568	7728
RXA00155	GR00023	8615	9397
RXA00162	GR00024	5438	5791
RXA00167	GR00025	4324	4584
RXA00169	GR00026	5222	5150
RXA00170	GR00026	9914	8081
RXA00171	GR00026	10318	10086
RXA00173	GR00027	1716	1384
RXA00174	GR00027	2079	1795
RXA00175	GR00027	2732	2103
RXA00176	GR00027	3475	3317
RXA00179	GR00028	1714	1258
RXA00194	GR00030	290	6
RXA00199	GR00031	2172	754
RXA00200	GR00031	2837	2535
RXA00207	GR00032	6430	6747
RXA00211	GR00032	10120	10782
RXA00218	GR00032	18104	19243
RXA00222	GR00032	21073	22218
RXA00230	GR00034	746	27

Identification	Code	NT	Stop
RXA00232	GR00035	527	18
RXA00236	GR00036	3300	2575
RXA00237	GR00036	3668	4045
RXA00238	GR00036	4186	4554
RXA00240	GR00036	5342	5133
RXA00242	GR00036	7031	8233
RXA00244	GR00037	1585	930
RXA00245	GR00037	3049	1565
RXA00250	GR00038	6	221
RXA00252	GR00038	485	727
RXA00255	GR00039	2	604
RXA00258	GR00039	988	1738
RXA00257	GR00039	1760	2215
RXA00258	GR00039	3219	3890
RXA00260	GR00039	9224	10409
RXA00261	GR00039	11693	11265
RXA00264	GR00040	2459	2836
RXA00267	GR00040	4091	3822
RXA00272	GR00041	4420	4791
RXA00273	GR00042	185	1297
RXA00274	GR00042	1555	4165
RXA00275	GR00042	4696	4238
RXA00276	GR00042	5016	4875
RXA00282	GR00044	793	5
RXA00283	GR00045	142	1289
RXA00286	GR00046	578	1142
RXA00284	GR00047	2761	3189
RXA00276	GR00049	2595	3416
RXA00302	GR00050	459	4
RXA00303	GR00050	1061	887
RXA00308	GR00052	358	537
RXA00320	GR00057	9378	9857
RXA00326	GR00057	16762	17097
RXA00334	GR00057		
RXA00337	GR00058	530	6
RXA00342	GR00061	73	501
RXA00320	GR00011	4013	5484
RXA00326	GR00011		
RXA00353	GR00068	988	1680
RXA00355	GR00069	635	510
RXA00357	GR00070	3724	2768
RXA00358	GR00070	4069	5199
RXA00362	GR00073	2	981
RXA00373	GR00079	342	4
RXA00375	GR00080	549	49
RXA00380	GR00082	836	216
RXA00384	GR00083	395	6
RXA00387	GR00084	1403	591
RXA00390	GR00086	1437	1841
RXA00392	GR00086	3890	3027

Identification	Code	NT	NT
Config.	Start	Stop	Stop
RXA00194	GR00086	4990	
RXA00195	CR00086	5716	
RXA00197	CR00086	6667	
RXA00198	GR00087	681	
RXA00198	GR00087	642	1088
RXA00199	GR00091	1088	2500
RXA00209	GR00091	1088	
RXA00213	GR00097	909	457
RXA00214	GR00097	1379	909
RXA00215	GR00097	1433	1657
RXA00219	GR00098	3063	2682
RXA00433	CR00100	1446	1970
RXA00451	GR00110	816	325
RXA00457	GR00114	1451	372
RXA00461	GR00116	4209	3388
RXA00468	GR00118	1282	464
RXA00469	GR00119	1647	472
RXA00472	GR00119	5449	4589
RXA00475	GR00119	8822	8163
RXA00476	GR00119	8861	9821
RXA00481	GR00119	17636	18220
RXA00486	GR00120	1	702
RXA00493	GR00123	3	326
RXA00496	GR00123	1776	2177
RXA00504	GR00125	5007	5252
RXA00507	GR00127	1098	244
RXA00509	GR00129	316	140
RXA00510	GR00129	384	914
RXA00519	GR00132	4	516
RXA00522	GR00134	111	575
RXA00527	GR00136	3121	1380
RXA00528	GR00138	3562	4650
RXA00529	GR00138	5274	4732
RXA00530	GR00136	6837	5557
RXA00535	GR00137	5155	5871
RXA00546	GR00142	1	690
RXA00547	GR00142	641	1054
RXA00548	GR00143	3	506
RXA00549	GR00143	502	897
RXA00550	GR00143	935	1255
RXA00554	GR00145	1606	1116
RXA00581	GR00151	1	2139
RXA00584	GR00151	3744	4148
RXA00576	GR00156	2916	2246
RXA00577	GR00156	2980	3327
RXA00582	GR00156	9442	8924
RXA00585	GR00156	11894	11577
RXA00589	GR00156	14220	14982
RXA00595	GR00159	3	332

Identification	Code	Config	Start	Stop	NT
RXA00597	GR00159	GR00159	797	1066	1066
RXA00598	GR00159	GR00159	1070	1387	1387
RXA00801	GR00159	GR00159	3459	3749	3749
RXA00804	GR00159	GR00159	5489	5779	5779
RXA00816	GR00162	GR00162	3574	3918	3918
RXA00817	GR00162	GR00162	4002	5084	5084
RXA00831	GR00166	GR00166	172	1626	1626
RXA00846	GR00169	GR00169	446	6	6
RXA00847	GR00169	GR00169	641	1271	1271
RXA00852	GR00169	GR00169	5449	5997	5997
RXA00853	GR00169	GR00169	6924	6160	6160
RXA00858	GR00169	GR00169	9495	9235	9235
RXA00861	GR00172	GR00172	684	1253	1253
RXA00882	GR00172	GR00172	2671	1403	1403
RXA00884	GR00173	GR00173	635	1219	1219
RXA00876	GR00178	GR00178	647	1393	1393
RXA00878	GR00179	GR00179	1037	103	103
RXA00892	GR00181	GR00181	3450	2317	2317
RXA00893	GR00181	GR00181	4303	3821	3821
RXA00701	GR00182	GR00182	427	801	801
RXA00704	GR00183	GR00183	2912	3484	3484
RXA00707	GR00185	GR00185	377	1348	1348
RXA00712	GR00187	GR00187	1048	500	500
RXA00714	GR00188	GR00188	1809	1249	1249
RXA00720	GR00188	GR00188	7665	7000	7000
RXA00721	GR00189	GR00189	381	5	5
RXA00723	GR00190	GR00190	537	4	4
RXA00724	GR00191	GR00191	811	164	164
RXA00725	GR00191	GR00191	458	808	808
RXA00728	GR00192	GR00192	841	701	701
RXA00729	GR00194	GR00194	1	642	642
RXA00730	GR00194	GR00194	1063	731	731
RXA00739	GR00202	GR00202	619	4	4
RXA00740	GR00202	GR00202	1646	1088	1088
RXA00741	GR00202	GR00202	2986	2054	2054
RXA00742	GR00202	GR00202	5517	3888	3888
RXA00743	GR00202	GR00202	6652	6230	6230
RXA00745	GR00202	GR00202	13874	11341	11341
RXA00748	GR00202	GR00202	13755	14945	14945
RXA00747	GR00202	GR00202	15087	15654	15654
RXA00748	GR00202	GR00202	15917	16360	16360
RXA00749	GR00202	GR00202	17240	16542	16542
RXA00750	GR00202	GR00202	18917	19314	19314
RXA00751	GR00202	GR00202	20245	19418	19418
RXA00752	GR00202	GR00202	21847	21419	21419
RXA00754	GR00203	GR00203	344	654	654
RXA00757	GR00203	GR00203	3119	4372	4372
RXA00769	GR00204	GR00204	6624	6836	6836

<u>Identification</u>	<u>NT</u>	<u>Stop</u>
<u>Code</u>	<u>Start</u>	<u>Config.</u>
RXA00771	GR00205	857
RXA00785	GR00207	625
RXA00788	GR00209	910
RXA00795	GR00211	4228
RXA00804	GR00215	438
RXA00811	GR00218	1695
RXA00812	GR00219	287
RXA00814	GR00219	246J
RXA00815	GR00219	3236
RXA00816	GR00219	4182
RXA00826	GR00221	567
RXA00831	GR00224	1662
RXA00838	GR00226	797
RXA00837	GR00227	540
RXA00840	GR00228	742
RXA00841	GR00228	1466
RXA00853	GR00231	3775
RXA00854	GR00231	4708
RXA00855	GR00232	526
RXA00862	GR00236	580
RXA00876	GR00241	4208
RXA00881	GR00242	8057
RXA00882	GR00242	8788
RXA00883	GR00242	10060
RXA00893	GR00244	769
RXA00895	GR00244	2578
RXA00904	GR00246	1457
RXA00908	GR00247	1611
RXA00914	GR00250	1271
RXA00915	GR00251	514
RXA00916	GR00251	4108
RXA00917	GR00251	5534
RXA00919	GR00252	1890
RXA00920	GR00252	2852
RXA00921	GR00252	4750
RXA00922	GR00252	6109
RXA00923	GR00252	6857
RXA00924	GR00252	7278
RXA00925	GR00252	8546
RXA00932	GR00253	5088
RXA00933	GR00253	6047
RXA00943	GR00258	J
RXA00946	GR00259	3034
RXA00959	GR00265	402
RXA00963	GR00269	442
RXA00969	GR00273	1
RXA00971	GR00273	1421
RXA00973	GR00274	2277

Identification	Card#	NT	NT
	Config	Start	Stop
RXA00978	GR00276	217	831
RXA00988	GR00280	1371	949
RXA01005	GR00286	520	1165
RXA01007	GR00287	2572	866
RXA01008	GR00287	2719	4859
RXA01016	GR00290	1141	494
RXA01028	GR00295	1	626
RXA01029	GR00295	1338	1826
RXA01031	GR00295	3182	3847
RXA01032	GR00295	3974	4348
RXA01033	GR00295	4363	4898
RXA01034	GR00295	5177	4824
RXA01035	GR00295	5816	6423
RXA01036	GR00295	6513	6965
RXA01037	GR00295	7000	7527
RXA01038	GR00295	7530	8276
RXA01039	GR00295	9540	8965
RXA01040	GR00295	9711	10613
RXA01041	GR00295	10780	10932
RXA01042	GR00295	11088	12385
RXA01043	GR00295	12774	13346
RXA01044	GR00295	14024	15280
RXA01045	GR00295	15407	17230
RXA01046	GR00295	17441	19219
RXA01047	GR00295	19244	19717
RXA01058	GR00296	8586	8248
RXA01062	GR00297	490	5
RXA01063	GR00297	828	499
RXA01066	GR00298	605	1330
RXA01069	GR00299	606	4
RXA01075	GR00300	3289	2859
RXA01083	GR00302	1777	1502
RXA01085	GR00303	980	4
RXA01086	GR00304	2	463
RXA01082	GR00305	702	881
RXA01098	GR00306	4341	3643
RXA01103	GR00306	10316	10092
RXA01107	GR00306	13612	14811
RXA01108	GR00306	15582	14912
RXA01109	GR00306	16281	15640
RXA01112	GR00307	1	870
RXA01121	GR00310	2479	3156
RXA01122	GR00311	557	38
RXA01123	GR00311	1090	844
RXA01127	GR00314	2	280
RXA01128	GR00314	1325	312
RXA01131	GR00315	445	1311
RXA01134	GR00317	2	460

Identification Code	Config.	NT Start	NT Stop
RXA01137	GR00318	1101	1480
RXA01140	GR00318	3272	4057
RXA01148	GR00321	1452	2051
RXA01153	GR00325	546	4
RXA01154	GR00326	808	6
RXA01155	GR00327	1170	6
RXA01158	GR00327	1588	1388
RXA01160	GR00328	4187	3213
RXA01161	GR00331	710	6
RXA01165	GR00332	2155	1583
RXA01168	GR00332	3005	2523
RXA01167	GR00333	3	323
RXA01169	GR00334	1	567
RXA01170	GR00334	638	1120
RXA01171	GR00334	1714	2408
RXA01173	GR00334	4853	4239
RXA01174	GR00334	6004	5255
RXA01178	GR00335	4106	4555
RXA01184	GR00338	1489	17
RXA01187	GR00338	3850	4308
RXA01206	GR00346	593	853
RXA01210	GR00349	3	895
RXA01211	GR00351	1508	282
RXA01218	GR00353	1078	1506
RXA01231	GR00356	1384	1887
RXA01233	GR00356	4242	3871
RXA01234	GR00357	631	250
RXA01255	GR00365	5613	5785
RXA01263	GR00387	10120	11631
RXA01287	GR00387	16799	15486
RXA01275	GR00387	28418	29335
RXA01276	GR00387	29893	30538
RXA01281	GR00389	3089	4630
RXA01295	GR00373	3764	4738
RXA01298	GR00373	5816	4754
RXA01301	GR00375	1993	1589
RXA01304	GR00376	1982	2467
RXA01308	GR00376	5691	4684
RXA01310	GR00380	803	477
RXA01313	GR00381	1116	172
RXA01315	GR00382	1394	744
RXA01316	GR00382	1855	1583
RXA01317	GR00382	2296	1877
RXA01326	GR00386	45	338
RXA01330	GR00387	569	1024
RXA01333	GR00389	1231	227
RXA01336	GR00389	1640	1038
RXA01337	GR00389	5085	3653

Identification Code	Config.	NT Start	NT Stop
RXA01149	GR00392	1531	755
RXA01176	GR00401	1281	4
RXA01181	GR00408	1147	5
RXA01184	GR00406	1228	1523
RXA01190	GR00408	992	336
RXA01191	GR00408	2078	1389
RXA01190	GR00409	2988	1489
RXA01401	GR00409	1193	1451
RXA01402	GR00409	3508	3981
RXA01403	GR00409	4410	3997
RXA01405	GR00410	1844	1389
RXA01410	GR00411	686	4
RXA01413	GR00412	854	1463
RXA01414	GR00412	1628	2134
RXA01415	GR00412	2192	4615
RXA01417	GR00414	645	49
RXA01421	GR00416	1215	829
RXA01422	GR00416	2003	1221
RXA01434	GR00417	10228	9063
RXA01440	GR00418	7496	8489
RXA01441	GR00418	8542	7514
RXA01445	GR00418	15083	14091
RXA01447	GR00418	17885	18743
RXA01448	GR00418	19796	19017
RXA01452	GR00419	2363	2641
RXA01456	GR00420	898	1419
RXA01457	GR00420	1499	2173
RXA01459	GR00420	3311	4120
RXA01460	GR00420	4068	4359
RXA01469	GR00422	2091	3122
RXA01470	GR00422	4112	3687
RXA01471	GR00422	5243	4437
RXA01472	GR00422	5783	5328
RXA01473	GR00422	6596	5832
RXA01474	GR00422	6878	7223
RXA01475	GR00422	7651	7226
RXA01476	GR00422	7847	8188
RXA01479	GR00422	12423	12650
RXA01484	GR00422	20868	19523
RXA01485	GR00422	20230	22281
RXA01518	GR00424	23238	23711
RXA01519	GR00424	23725	24471
RXA01520	GR00424	24784	25167
RXA01525	GR00424	32301	30580
RXA01527	GR00425	5126	2616
RXA01529	GR00426	2	277
RXA01546	GR00427	4086	2825
RXA01539	GR00428	120	2042

Identification	Code	NT	Stop
NT	Start	Contig	Contig
RXA01540	GR000428	3081	2382
RXA01542	GR000429	3	5063
RXA01543	GR000430	2802	37
RXA01544	GR000430	1496	2897
RXA01545	GR000430	4838	3588
RXA01546	GR000430	5584	4889
RXA01547	GR000430	6371	5709
RXA01548	GR000430	7432	6425
RXA01549	GR000430	8426	7586
RXA01552	GR000431	6122	5145
RXA01554	GR000432	7119	1578
RXA01557	GR000433	859	1774
RXA01560	GR000435	767	438
RXA01565	GR000437	1176	708
RXA01566	GR000437	1668	1202
RXA01567	GR000437	2213	1614
RXA01574	GR000438	6963	5929
RXA01575	GR000438	8024	7005
RXA01579	GR000439	671	1054
RXA01586	GR000441	1597	1229
RXA01587	GR000442	120	2102
RXA01590	GR000445	1710	427
RXA01596	GR000447	7414	8376
RXA01602	GR000447	13591	12082
RXA01605	GR000448	880	2474
RXA01610	GR000449	4343	3615
RXA01611	GR000449	4832	4476
RXA01612	GR000449	5235	4891
RXA01618	GR000451	1387	1004
RXA01619	GR000451	2407	1403
RXA01627	GR000453	1	495
RXA01628	GR000453	866	1879
RXA01630	GR000454	341	1417
RXA01634	GR000454	4988	5539
RXA01638	GR000456	825	436
RXA01639	GR000456	1334	897
RXA01641	GR000456	5182	6552
RXA01642	GR000456	6557	7798
RXA01643	GR000456	8374	7949
RXA01652	GR000458	971	6
RXA01659	GR000462	3	488
RXA01663	GR000463	438	4
RXA01665	GR000463	2152	1433
RXA01672	GR000467	2	310
RXA01675	GR000467	2824	3234
RXA01676	GR000467	4179	3424
RXA01681	GR000467	10681	11313
RXA01688	GR000470	2026	1586

Identification	Code	NT	Stop
	Config	Start	
RXA01694	GR00474	391	3032
RXA01697	GR00476	761	1486
RXA01701	CR00476	196	528
RXA01703	CR00479	2118	1648
RXA01708	CR00482	312	602
RXA01711	GR00484	2007	850
RXA01714	GR00485	985	371
RXA01729	GR00489	2636	3154
RXA01731	CR00491	109	807
RXA01734	GR00492	544	1077
RXA01741	GR00493	7535	6738
RXA01742	GR00493	7614	8117
RXA01750	GR00496	1878	3518
RXA01751	GR00496	5576	5830
RXA01752	GR00497	557	6
RXA01753	GR00497	2095	557
RXA01754	GR00497	4082	2142
RXA01760	GR00498	5095	5376
RXA01761	GR00499	7001	5484
RXA01765	GR00500	3144	4085
RXA01767	GR00501	341	6
RXA01768	GR00501	827	450
RXA01769	GR00501	1275	847
RXA01770	GR00501	5134	1370
RXA01771	GR00502	886	185
RXA01773	CR00503	14	444
RXA01774	CR00503	634	1416
RXA01775	GR00504	176	741
RXA01776	GR00504	838	2289
RXA01777	GR00504	2319	2777
RXA01778	GR00504	2912	4048
RXA01779	GR00504	4246	5884
RXA01780	GR00504	5721	6095
RXA01781	GR00504	6052	6312
RXA01782	GR00504	6384	6779
RXA01783	GR00504	6842	7078
RXA01785	GR00505	729	1304
RXA01787	GR00505	2	155
RXA01788	GR00506	361	801
RXA01789	GR00506	875	1516
RXA01790	GR00506	1672	1731
RXA01791	GR00506	1885	2247
RXA01792	GR00506	2310	2582
RXA01793	GR00506	2916	3149
RXA01794	GR00506	3194	3427
RXA01799	GR00509	377	1570
RXA01800	GR00509	2292	1573
RXA01809	GR00510	3	638

<u>Identification</u>	<u>Code</u>	<u>Config</u>	<u>Start</u>	<u>NT</u>	<u>Stop</u>
RXA01812	GR00514	3	1232		
RXA01813	GR00515	635	6		
RXA01816	GR00515	4210	4941		
RXA01817	GR00515	4941	5573		
RXA01820	GR00515	8380	9733		
RXA01825	GR00516	2847	2578		
RXA01831	GR00516	10874	10413		
RXA01834	GR00517	2478	1777		
RXA01842	GR00522	1397	480		
RXA01843	GR00522	876	1067		
RXA01845	GR00522	1919	2326		
RXA01846	GR00523	261	4		
RXA01847	GR00524	52	786		
RXA01854	GR00525	5692	5946		
RXA01855	GR00526	1	1818		
RXA01856	GR00527	225	770		
RXA01857	GR00527	939	1589		
RXA01858	GR00529	678	6		
RXA01870	GR00534	2123	2797		
RXA01874	GR00535	2556	2803		
RXA01899	GR00544	1874	2859		
RXA01902	GR00544	7957	7094		
RXA01903	GR00545	3	281		
RXA01904	GR00545	762	340		
RXA01905	GR00545	1074	1604		
RXA01906	GR00545	2322	2788		
RXA01907	GR00545	3176	3787		
RXA01908	GR00545	4030	4612		
RXA01909	GR00546	59	937		
RXA01910	GR00546	1030	1875		
RXA01911	GR00546	2189	3044		
RXA01921	GR00551	943	5		
RXA01923	GR00552	1311	1739		
RXA01924	GR00553	1	817		
RXA01925	GR00553	1008	1674		
RXA01930	GR00555	2817	2867		
RXA01941	GR00557	995	1429		
RXA01958	GR00563	221	1270		
RXA01957	GR00564	889	850		
RXA01958	GR00564	910	1416		
RXA01959	GR00564	1639	2019		
RXA01980	GR00585	187	504		
RXA01981	GR00585	521	1000		
RXA01982	GR00585	1022	1591		
RXA01983	GR00585	1757	2440		
RXA01984	GR00586	1329	4		
RXA01985	GR00586	1935	1375		
RXA01989	GR00587	5889	5216		

Identification	Code	NT	Start	Stop
Config.	Config.	NT	Start	Stop
RXA01973	GR00570	583	2108	
RXA01974	GR00570	658	2108	
RXA01976	GR00571	3742	2222	
RXA01977	GR00571	4647	3972	
RXA01978	GR00572	1	1167	
RXA01981	GR00573	2105	2583	
RXA01987	GR00576	167	179	
RXA01888	GR00576	779	462	
RXA01990	GR00581	1	999	
RXA01991	GR00581	926	1720	
RXA01999	GR00589	2384	2854	
RXA02001	GR00589	700	152	
RXA02003	GR00593	501	4	
RXA02004	GR00594	3	209	
RXA02005	GR00594	166	447	
RXA02006	GR00597	498	4	
RXA02007	GR00598	651	223	
RXA02009	GR00601	127	5	
RXA02011	GR00603	46	363	
RXA02013	GR00607	553	5	
RXA02014	GR00607	935	540	
RXA02019	GR00612	597	106	
RXA02021	GR00613	2008	1061	
RXA02036	GR00619	3441	3821	
RXA02039	GR00621	3	812	
RXA02040	GR00621	1452	925	
RXA02045	GR00623	1913	2173	
RXA02046	GR00623	2680	2943	
RXA02049	GR00624	1583	2029	
RXA02050	GR00624	2462	2833	
RXA02051	GR00624	3188	3683	
RXA02053	GR00624	5484	6062	
RXA02058	GR00625	4051	5500	
RXA02059	GR00625	4878	4184	
RXA02066	GR00626	6187	6678	
RXA02067	GR00626	6713	7188	
RXA02069	GR00627	1116	1694	
RXA02081	GR00628	12307	13905	
RXA02084	GR00629	2920	2576	
RXA02089	GR00629	8431	8901	
RXA02090	GR00629	9764	8984	
RXA02091	GR00629	10512	9862	
RXA02094	GR00629	13282	13998	
RXA02097	GR00630	184	3555	
RXA02102	GR00631	4479	3122	
RXA02103	GR00631	4510	4905	
RXA02109	GR00632	3480	2540	
RXA02114	GR00634	615	120	

Table 1. Page 20

Identification	Code	NT	Stop
	Config.	NT	Start
RXA02121	GR00636	5813	5109
RXA02125	GR00637	739	1519
RXA02129	GR00637	5906	6139
RXA02146	GR00639	14742	15168
RXA02151	GR00639	18913	21100
RXA02152	GR00640	237	638
RXA02163	GR00640	10072	10824
RXA02164	GR00640	10824	12398
RXA02165	GR00640	12988	12999
RXA02168	GR00641	2894	81
RXA02169	GR00641	3172	4017
RXA02170	GR00641	4798	4025
RXA02178	GR00641	13628	14497
RXA02181	GR00641	17168	17845
RXA02185	GR00641	20185	20761
RXA02186	GR00641	21213	20995
RXA02199	GR00646	2591	3160
RXA02203	GR00646	7469	7092
RXA02208	GR00848	9827	10862
RXA02207	GR00646	10909	11667
RXA02212	GR00649	964	467
RXA02221	GR00646	6720	8081
RXA02226	GR00651	1059	4
RXA02227	GR00651	1216	1853
RXA02220	GR00653	4156	3620
RXA02231	GR00851	5111	4356
RXA02238	GR00654	5241	5525
RXA02256	GR00855	653	1185
RXA02267	GR00855	2053	1181
RXA02271	GR00855	5406	5983
RXA02279	GR00657	1	1404
RXA02280	GR00658	2	754
RXA02281	GR00660	2	532
RXA02285	GR00860	1544	2272
RXA02286	GR00860	3285	3833
RXA02287	GR00860	4071	4872
RXA02294	GR00862	5992	5618
RXA02296	GR00862	6978	7466
RXA02300	GR00862	11184	10862
RXA02301	GR00862	11910	11194
RXA02302	GR00862	12036	12800
RXA02303	GR00863	1	720
RXA02304	GR00863	1613	723
RXA02307	GR00864	395	6
RXA02325	GR00868	4314	3445
RXA02330	GR00870	605	15
RXA02331	GR00871	396	761
RXA02336	GR00872	2731	2552

Identification Code	NT Config.	NT Start	NT Stop
RXA02338	GR00673	484	5
RXA02339	GR00674	1	492
RXA02340	GR00674	1214	576
RXA02341	GR00675	415	5
RXA02356	GR00684	761	1756
RXA02358	GR00685	1239	1529
RXA02360	GR00685	1644	6076
RXA02361	GR00685	6160	6810
RXA02362	GR00685	7045	10743
RXA02366	GR00687	254	1581
RXA02368	GR00687	2918	2244
RXA02374	GR00688	1626	2246
RXA02381	GR00691	1792	770
RXA02398	GR00698	2841	4370
RXA02401	GR00699	3391	4491
RXA02406	GR00701	1322	774
RXA02412	GR00703	2043	2522
RXA02415	GR00704	655	170
RXA02417	GR00705	4755	2632
RXA02421	GR00705	7237	6428
RXA02423	GR00706	221	6
RXA02428	GR00707	4585	3452
RXA02433	GR00708	2981	3580
RXA02437	GR00708	1661	2470
RXA02444	GR00709	7818	9113
RXA02454	GR00711	3	815
RXA02457	GR00712	1295	2404
RXA02480	GR00712	5839	5336
RXA02481	GR00712	6252	5845
RXA02484	GR00713	1107	1613
RXA02485	GR00713	2014	1616
RXA02486	GR00714	92	6
RXA02487	GR00714	843	419
RXA02473	GR00715	6864	5924
RXA02475	GR00715	9585	8441
RXA02476	GR00716	1245	10
RXA02483	GR00718	1813	1001
RXA02498	GR00720	11016	11819
RXA02500	GR00720	13480	13558
RXA02505	GR00720	18413	18597
RXA02506	GR00720	19464	18663
RXA02510	GR00721	1983	2818
RXA02519	GR00724	1913	128
RXA02520	GR00724	2222	2905
RXA02534	GR00726	5536	6339
RXA02537	GR00726	8961	9422
RXA02538	GR00726	9422	10093
RXA02546	GR00728	19927	16824

Identification Code	NT	Stop
Config.	Start	
RXA02552	GR00730	110
RXA02554	GR00731	427
RXA02555	GR00731	1155
RXA02564	GR00732	2543
RXA02568	GR00735	1363
RXA02569	GR00736	5
RXA02570	GR00736	82
RXA02576	GR00740	831
RXA02577	GR00740	1478
RXA02591	GR00741	1569
RXA02593	GR00741	1569
RXA02594	GR00741	1569
RXA02606	GR00742	148
RXA02609	GR00740	2463
RXA02610	GR00741	1579
RXA02619	GR00741	1579
RXA02620	GR00746	1579
RXA02624	GR00746	1579
RXA02647	GR00742	1579
RXA02649	GR00746	1579
RXA02652	GR00746	1579
RXA02655	GR00752	1579
RXA02682	GR00753	1579
RXA02670	GR00753	1579
RXA02673	GR00753	1579
RXA02678	GR00754	1579
RXA02679	GR00754	1579
RXA02680	GR00754	1579
RXA02681	GR00754	1579
RXA02683	GR00754	1579
RXA02685	GR00754	1579
RXA02696	GR00756	1579
RXA02712	GR00756	1579
RXA02715	GR00756	1579
RXA02725	GR00760	1579
RXA02727	GR00760	1579
RXA02734	GR00762	1579
RXA02736	GR00763	1579
RXA02744	GR00763	1579
RXA02753	GR00765	1579
RXA02758	GR00766	1579
RXA02757	GR00766	1579
RXA02765	GR00769	1579
RXA02770	GR00772	1579
RXA02774	GR00773	1579
RXA02775	GR00773	1579
RXA02776	GR00773	1579
RXA02778	GR00773	1579
RXA0277	GR00773	1579

Identification	Code	NT	Stop
RXA02778	GR00773	10095	10319
RXA02779	GR00773	10617	10895
RXA02780	GR00773	10954	11280
RXA02781	GR00774	1145	155
RXA02782	GR00775	204	875
RXA02783	GR00775	845	1393
RXA02784	GR00775	1751	1936
RXA02786	GR00777	2	808
RXA02793	GR00777	9185	8684
RXA02812	GR00780	2	568
RXA02815	GR00798	3	554
RXA02816	GR00797	2	499
RXA02817	GR00798	403	5
RXA02818	GR00799	611	6
RXA02823	GR00804	275	6
RXA02825	GR00806	585	182
RXA02827	GR00812	428	6
RXA02835	GR00824	289	523
RXA02838	GR00831	1	462
RXA02841	GR00840	283	5
RXA02842	GR00841	356	15
RXA02844	GR00843	247	495
RXA02845	GR00844	2	616
RXA02846	GR00845	570	6
RXA02848	CR10003	459	211
RXA02849	CR10004	1392	287
RXA02862	CR10006	1695	2330
RXA02867	CR10008	610	5
RXA02868	CR10009	2017	1262
RXA02869	CR10009	390	4
RXA02870	GR10011	6	344
RXA02871	GR10011	398	830
RXA02876	GR10016	405	1067
RXA02881	GR10019	94	759
RXA02882	GR10020	2	724
RXA02885	GR10021	1	1538
RXA02886	GR10024	328	754
RXA02889	GR10026	1123	2706
RXA02891	GR10035	1	602
RXA02892	GR10035	1171	668
RXA02896	GR10038	256	5
RXA02903	GR10044	477	4
RXA01494	GR00423	8515	7520
RXA01092	GR00305	702	881
RXA01186	GR00338	3742	2845

TABLE 2: GENES IDENTIFIED FROM GENBANK

GenBank™ Accession No.	Gene Name	Gene Function	Reference
A09073	ppg	Phosphoenol pyruvate carboxylase	Bachmann, B. et al. "DNA fragment coding for phosphoenolpyruvate carboxylase, recombinant DNA carrying said fragment, strains carrying the recombinant DNA and method for producing L-amino acids using said strains," Patent: EP 0358940 A 3 03/21/90
A45579, A45581, A45583, A45585		Threonine dehydratase	Moekkel, B. et al. "Production of L-isoleucine by means of recombinant micro-organisms with deregulated threonine dehydratase," Patent. WO 9519442 A 5 07/20/95
A45587 AB003132	murC, flsQ, flsZ		Kobayashi, M. et al. "Cloning, sequencing and characterization of the flsZ gene from coryneform bacteria," <i>Biochem. Biophys. Res. Commun.</i> , 236(2):383-388 (1997)
AB015023	murC, flsQ		Wachi, M. et al. "A murC gene from Coryneform bacteria," <i>Appl. Microbiol. Biotechnol.</i> , 51(2):223-228 (1999)
AB018530	disR		Kimura, F. et al. "Molecular cloning of a novel gene, disR, which rescues the detergent sensitivity of a mutant derived from <i>Brevibacterium lactosfermentum</i> ," <i>Biochi. Biotechnol. Biotech.</i> , 60(10):1565-1570 (1996)
AB018531	disR1, disR2	D-glutamate racemase	
AB020624	murI	Transketolase	
AB023377	rkI	Glutamine 2-oxoglutarate aminotransferase	
AB024708	gltB, gltD	large and small subunits	
AB02524	acn	aconitase	
AB027714	rep	Replication protein	
AB027715	rep, aad	Replication protein, aminoglycoside acetyltransferase	
AF005242	angC	N-acetylglutamate- β -semialdehyde dehydrogenase	
AF005635	glnA	Glutamine synthetase	
AF030405	hisF	cyclase	
AF030520	argG	Alphinosuccinate synthetase	
AF031518	argF	Ornithine carbamoyltransferase	
AF036932	atD	3-dehydroquinic acid hydrolase	

GenBank™ Accession No.	Gene Name	Gene Function	Reference
AF038548	pyc	Pyruvate carboxylase	Wachmeier, L. et al. "The role of the <i>Corynebacterium glutamicum</i> <i>tel</i> gene in (P)ppGpp metabolism." <i>Microbiology</i> , 144, 1853-1862 (1998)
AF038651	dttE; pti; rt	Dipeptide-binding protein; adenosine phosphoribosyltransferase; GTP pyrophosphokinase	
AF041436	argR	Arginine repressor	
AF045998	impA	Inositol monophosphate phosphatase	
AF048764	argH	Argininosuccinate lyase	
AF049897	argC; argI; argB; argD; argF; argR; argG; argH	N-acetylglutamylphosphate racyclase; N-ornithine acetyltransferase; N-acetylglutamate kinase; acetylornithine transimine; ornithine acetyltransferase; arginine repressor; carbamoyltransferase; argininosuccinate synthase; argininosuccinate lyase	
AF050109	inhA	Enoyl-acyl carrier protein reductase	
AF050166	hisG	ATP phosphoribosyltransferase	
AF051846	hisA	Phosphoribosylformimino- δ -amino-1-phosphoribosyl-4-imidazole acboxamidic isomerase	
AF052652	metA	Homocysteine O-acetyltransferase	Park, S. et al. "Isolation and analysis of <i>metA</i> , a methionine biosynthetic gene encoding homocysteine acetyltransferase in <i>Corynebacterium glutamicum</i> ." <i>Microbiology</i> , 144, 286-294 (1998)
AF053071	aroB	Dihydroquinolale synthetase	
AF060558	hisH	Glutaminc amidotransferase	
AF086704	hisE	Phosphoribosyl- Δ TP pyrophosphohydrolase	
AF114233	aroA	S-enolpyruvylshikimate 3-phosphate synthase	
AF116184	panD	L-aspartate- α -alpha-decarboxylase precursor overproduction in <i>Escherichia coli</i> ." <i>Appl. Environ. Microbiol.</i> , 65(4) 1530-1539 (1999)	Dusch, N. et al. "Expression of the <i>Corynebacterium glutamicum</i> <i>panD</i> gene encoding L-aspartate- α -decarboxylase leads to pantothenate

GenBank™ Accession No.	Gene Name	Gene Function	Reference
AF124518	aroD; aroE	3-dehydroquinate; shikimate dehydrogenase	
AF124600	aroC; aroK; aroB; pepQ	Chorismate synthase; shikimate kinase; 3-dehydroquinate synthase; putative cytoplasmic peptidase	
AF145897	inhA		
AF145898	inhA	Transpun of ectoine, glycine betaine, proline	Peter, H. et al. "Corynebacterium glutamicum is equipped with four secondary carriers for compatible solutes. Identification, sequencing, and characterization of the proline/ectoine uptake system, ProP, and the ectoine/proline/glycine betaine carrier, EcpP." <i>J. Bacteriol.</i> , 180(22):6005-6012 (1998)
AJ001436	ectP		Wehrmann, A. et al. "Different modes of diaminopimelate synthesis and their role in cell wall integrity: A study with Corynebacterium glutamicum." <i>J. Bacteriol.</i> , 180(12):3159-3165 (1998)
AJ004934	dapD	Tetrahydrodipicolinate succinylase (incomplete)	
AJ007732	ppc; scrG; amt; ocd; soxA	Phosphoenolpyruvate-carboxylase; ?, high affinity ammonium uptake protein; putative ornithine-cyclodecarboxylase; saucosine oxidase	Jakoby, M. et al. "Nitrogen regulation in Corynebacterium glutamicum: Isolation of genes involved in biochemical characterization of corresponding proteins." <i>FEMS Microbiol.</i> , 173(2):303-310 (1999)
AJ010319	fisY; glnB; glnD; sfp; amIP	Involved in cell division: PII protein; uridylyltransferase (uridylyl-removing enzyme); signal recognition particle; low affinity ammonium uptake protein	
AJ132968	cal	Chloramphenicol acetyl transferase	
AJ224946	mqo	L-malate: quinone oxidoreductase	Molenhaar, D. et al. "Biochemical and genetic characterization of the membrane-associated malate dehydrogenase (acepD) from Corynebacterium glutamicum." <i>Eur. J. Biochem.</i> , 254(2):395-403 (1998)
AJ239250	ndh	NADH dehydrogenase	Lichinger, T. et al. "Biochemical and biophysical characterization of the cell wall protein of Corynebacterium glutamicum: The channel is formed by a low molecular mass polypeptide." <i>Biochemistry</i> , 37(43):15024-15032 (1998)
AJ238703	polA	Ponin	Veres, A. A. et al. "Isolation and characterization of IS31831, a transposable element from Corynebacterium glutamicum." <i>Mol. Microbiol.</i> , 11(4):739-746 (1994)
D17429		Transposable element IS31831	

GenBank TM Accession No.	Gene Name	Gene Function	Reference
D84102	odhA	2-oxoglutarate dehydrogenase	Usuda, Y. et al. "Molecular cloning of the <i>Corynebacterium glutamicum</i> (Brevibacterium lactofermentum A1) odhA gene encoding a novel type of 2-oxoglutarate dehydrogenase," <i>Microbiology</i> 142:3347-3354 (1996)
E01358	hth, lk	Lhomosine dehydrogenase; homosine kinase	Katsunuma, R. et al. "Production of L-threonine and L-isoleucine," Patent: JP 1987232392-A 1 10/12/87
E01359		Upstream of the start codon of homoserine kinase gene	Katsunuma, R. et al. "Production of L-threonine and L-isoleucine," Patent: JP 1987232392-A 2 10/12/87
E01375	trpL; trpE	Tryptophan operon Leader peptide; anthranilate synthase	Matsui, K. et al. "Tryptophan operon, peptide and protein coded thereby, utilization of tryptophan operon gene expression and production of tryptophan," Patent: JP 1987244382-A 1 10/24/87
E01376		Promoter and operator regions of tryptophan operon	Matsui, K. et al. "Tryptophan operon, peptide and protein coded thereby, utilization of tryptophan operon gene expression and production of tryptophan," Patent: JP 1987244382-A 1 10/24/87
E01377		Biotin synthase	Hatakeyama, K. et al. "DNA fragment containing gene capable of coding biotin synthetase and its utilization," Patent: JP 1992278088-A 1 10/02/92
E03937		Diamino pelargonic acid aminotransferase	Kohama, K. et al. "Gene coding diamino pelargonic acid aminotransferase and desthiobiotin synthetase and its utilization," Patent: JP 1992330284-A 1 11/18/92
E04040		Desthiobiotin synthetase	Kohama, K. et al. "Gene coding diamino pelargonic acid aminotransferase and desthiobiotin synthetase and its utilization," Patent: JP 1992330284-A 1 11/18/92
E04041		Flavum aspartase	Kurusu, Y. et al. "Gene DNA coding aspartase and utilization thereof," Patent: JP 1993030977-A 1 02/09/93
E04307		Isocitric acid lyase	Katsunuma, R. et al. "Gene manifestation controlling DNA," Patent: JP 1993036782-A 3 03/09/93
E04376		Isocitric acid lyase N-terminal fragment	Katsunuma, R. et al. "Gene manifestation controlling DNA," Patent: JP 1993036782-A 3 03/09/93
E04377		Phenylate dehydratase	Solonchi, N. et al. "Production of L-phenylalanine by fermentation," Patent: JP 1993076152-A 2 03/30/93
E04484		Aspartokinase	Fujimoto, N. et al. "Gene DNA coding Aspartokinase and its use," Patent: JP 1993184366-A 1 07/27/93
E05108		Dihydro-dipicolarinate synthetase	Hatakeyama, K. et al. "Gene DNA coding dihydro-dipicolarinic acid synthetase and its use," Patent: JP 1993184371-A 1 07/27/93
E05112			

GenBank™ Accession No.	Gene Name	Gene Function	Reference
E05776	Diaminopimelic acid dehydrogenase	Kobayashi, M. et al. "Gene DNA coding Diaminopimelic acid dehydrogenase and its use," Patent. JP 199328970-A 1 11/02/93	
E05779	Threonine synthase	Kohama, K. et al. "Gene DNA coding threonine synthase and its use," Patent. JP 1993284972-A 1 11/02/93	
E06110	Prephenate dehydratase	Kikuchi, T. et al. "Production of L-phenylalanine by fermentation method," Patent. JP 1993344881-A 1 12/27/93	
E06111	Mutated Prephenate dehydratase	Kikuchi, T. et al. "Production of L-phenylalanine by fermentation method," Patent. JP 1993344881-A 1 12/27/93	
E06146	Acetohydroxy acid synthetase	Inui, M. et al. "Gene capable of coding Acetohydroxy acid synthetase and its use," Patent. JP 1993344891-A 1 12/27/93	
E06825	Aspartokinase	Sugimoto, M. et al. "Mutant aspartokinase gene," Patent. JP 1994062866-A 1 03/08/94	
E06826	Mutated aspartokinase alpha subunit	Sugimoto, M. et al. "Mutant aspartokinase gene," Patent. JP 1994062866-A 1 03/08/94	
E06827	Mutated aspartokinase alpha subunit	Sugimoto, M. et al. "Mutant aspartokinase gene," Patent. JP 1994062866-A 1 03/08/94	
E07701	sccY	Honno, N. et al. "Gene DNA participating in integration of membranous protein to membrane," Patent. JP 199416970-A 1 06/11/94	
E08177	Aspartokinase	Sato, Y. et al. "Genetic DNA capable of coding Aspartokinase released from feedback inhibition and its utilization," Patent. JP 1994261766-A 1 09/20/94	
E08178, E08179, E08180, E08181, E08182	Feedback inhibition-released Aspartokinase	Sato, Y. et al. "Genetic DNA capable of coding Aspartokinase released from feedback inhibition and its utilization," Patent. JP 1994261766-A 1 09/20/94	
E08232	Acetohydroxy-acid isomeroeductase	Inui, M. et al. "Gene DNA coding acetohydroxy acid isomeroeductase," Patent. JP 1994277067-A 1 10/04/94	
E08234	sccE	Asai, Y. et al. "Gene DNA coding for translocation machinery of protein," Patent. JP 1994277073-A 1 10/04/94	
E08443	F7 aminotransferase and desulphobiotin synthase promoter region	Iitakayama, K. et al. "DNA fragment having promoter function in coryneform bacterium," Patent. JP 1995031476-A 1 02/03/95	
E08446	Biotin synthetase	Iitakayama, K. et al. "DNA fragment having promoter function in coryneform bacterium," Patent. JP 1995031476-A 1 02/03/95	

Table 2, Page 5

GenBank™ Accession No.	Gene Name	Gene Function	Reference
E08649	Aspartase	Kohama, K. et al "DNA fragment having promoter function in coryneform bacterium," Patent. JP 1995031478-A 1 02/03/95	
E08900	Dihydrodipicolinate reductase	Medori, M. et al "DNA fragment containing gene coding Dihydrodipicolinate acid reductase and utilization thereof," Patent. JP 1995075578-A 1 03/20/95	
E08901	Diaminopimelic acid decarboxylase	Medori, M. et al "DNA fragment containing gene coding Diaminopimelic acid decarboxylase and utilization thereof," Patent. JP 1995075579-A 1 03/20/95	
E12594	Serine hydroxymethyltransferase	Halakayama, K. et al. "Production of L-hydrophan," Patent. JP 1997028391-A 1 02/04/97	
E12760, E12759, E12758	transposase	Moriya, M. et al "Amplification of gene using artificial transposon," Patent. JP 1997070291-A 03/18/97	
E12764	Arginyl-tRNA synthetase; diaminopimelic acid decarboxylase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent. JP 1997070291-A 03/18/97	
E12767	Dihydrodipicolinic acid synthetase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent. JP 1997070291-A 03/18/97	
E12770	aspartokinase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent. JP 1997070291-A 03/18/97	
E12773	Dihydrodipicolinic acid reductase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent. JP 1997070291-A 03/18/97	
E13655	Glucose-6-phosphate dehydrogenase	Halakayama, K. et al. "Glucose-6-phosphate dehydrogenase and DNA capable of coding the same," Patent. JP 1997224661-A 1 09/02/97	
L01508	IlvA	Morckel, B. et al. "Functional and structural analysis of the threonine dehydratase of <i>Corynebacterium glutamicum</i> ," <i>J. Bacteriol.</i> , 174:8065-8072 (1992)	
L07603	EC 4.2.1.15	Chen, C. et al. "The cloning and nucleotide sequence of <i>Corynebacterium glutamicum</i> 3-deoxy-D-arabinohexulosonate-7-phosphate synthase gene," <i>FEBS Microbiol Lett.</i> , 107:223-230 (1993)	
L09232	IlvB; ilvN; ilvC	Keilhauer, C. et al. "Isopelcic acid synthesis in <i>Corynebacterium glutamicum</i> . molecular analysis of the ilvB-ilvN-ilvC operon," <i>J. Bacteriol.</i> , 175(17):5595-5603 (1993)	

GenBank™ Accession No.	Gene Name	Gene Function	Reference
L18874	psm	Phosphoenolpyruvate sugar phosphotransferase	Fouet, A et al. "Bacillus subtilis sucrose-specific enzyme II of the phosphotransferase system: expression in <i>Escherichia coli</i> and homology to enzymes II from enteric bacteria," <i>PNAS USA</i> , 84(24): 8773-8777 (1987); Lee, J.K. et al. "Nucleotide sequence of the gene encoding the <i>Corynebacterium glutamicum</i> mannose enzyme II and analyses of the deduced protein sequence," <i>FEMS Microbiol. Lett.</i> , 119(1-2): 137-145 (1994)
L27123	aceB	Malate synthase	Lee, H-S. et al. "Molecular characterization of aceB, a gene encoding malate synthase in <i>Corynebacterium glutamicum</i> ," <i>J. Microbiol. Biotechnol.</i> , 4(4): 256-263 (1994)
L27126		Pyruvate kinase	Jelien, M. S. et al. "Structural and functional analysis of pyruvate kinase from <i>Corynebacterium glutamicum</i> ," <i>Appl. Environ. Microbiol.</i> , 60(7): 2501-2507 (1994)
L28760	aceA	Isocitrate lyase	Ogurza, J.A. et al. "Molecular cloning, DNA sequence analysis, and characterization of the <i>Corynebacterium diphtheriae</i> dtrR from <i>Brevibacterium lacfermentum</i> ," <i>J. Bacteriol.</i> , 177(2): 465-467 (1995)
L35906	dtrR	Diphtheria toxin repressor	Follette, M.T. et al. "Molecular cloning and nucleotide sequence of the <i>Corynebacterium glutamicum</i> phcA gene," <i>J. Bacteriol.</i> , 167:695-702 (1986)
M13774		Phenylalanyl dehydrodialase	Park, Y-H. et al. "Phylogenetic analysis of the coryneform bacteria by 5S rRNA sequences," <i>J. Bacteriol.</i> , 169:1801-1806 (1987)
M16175	5S rRNA		Sano, K. et al. "Structure and function of the trp operon control regions of <i>Brevibacterium lacfermentum</i> , a glutamic-acid producing bacterium," <i>Gene</i> , 52:191-200 (1987)
M16663	trpE	Anthranilate synthase, 5' end	Sano, K. et al. "Structure and function of the trp operon control regions of <i>Brevibacterium lacfermentum</i> , a glutamic-acid producing bacterium," <i>Gene</i> , 52:191-200 (1987)
M16664	trpA	Tryptophan synthase, 3' end	O'Regan, M. et al. "Cloning and nucleotide sequence of the Phosphoenolpyruvate carboxylase-coding gene of <i>Corynebacterium glutamicum</i> ATCC13032," <i>Gene</i> , 77(2):237-251 (1989)
M25819		Phosphoenolpyruvate carboxylase	Roller, C. et al. "Gram-positive bacteria with a high DNA G+C content are characterized by a common insertion within their 23S rRNA genes," <i>J. Gen. Microbiol.</i> , 138:1167-1175 (1992)
M85106		23S rRNA gene insertion sequence	

GenBank™ Accession No.	Gene Name	Gene Function	Reference
M85107, M85108	23S rRNA gene insertion sequence		Roller, C. et al. "Gram-positive bacteria with a high DNA G+C content are characterized by a common insertion within their 23S rRNA genes," <i>J. Gen Microbiol.</i> , 138: 1167-1175 (1992)
M89931	aceD; bmoQ; yhhw	Beta C-S lyase, branched-chain amino acid uptake carrier, hypothetical protein yhhw	Rossol, I. et al. "The <i>Corynebacterium glutamicum</i> aceD gene encodes a C-S lyase with alpha, beta-elimination activity that degrades aminothiylcysteine," <i>J. Bacteriol.</i> , 174(9):2968-2977 (1992); Tauch, A. et al. "Isoleucine uptake in <i>Corynebacterium glutamicum</i> ATCC 13032 is directed by the bmoQ gene product," <i>Arch. Microbiol.</i> , 169(4):303-312 (1998)
S59299	trpP	LacZα gene (promoter)	Henry, D.M. et al. "Cloning of the trp gene cluster from a tryophan-hyperproducing strain of <i>Corynebacterium glutamicum</i> : identification of a mutation in the trp leader sequence," <i>Appl. Environ. Microbiol.</i> , 59(3):791-799 (1993)
U11545	trpD	Anthranilate phosphoribosyltransferase	O'Gara, J.P. and Duncan, L.K. (1994) Complete nucleotide sequence of the <i>Corynebacterium glutamicum</i> ATCC 21850 trpD gene." Thesis, Microbiology Department, University College Galway, Ireland.
U13922	cglM; cglR; cglT	Putative type II S-cysteine methyltransferase; putative type II restriction endonuclease; putative type I or type III restriction endonuclease	Schäfer, A. et al. "Cloning and characterization of a DNA region encoding a stress-sensitive restriction system from <i>Corynebacterium glutamicum</i> ATCC 13032 and analysis of its role in intergeneric conjugation with <i>Escherichia coli</i> ," <i>J. Bacteriol.</i> , 176(23):7309-7319 (1994); Schäfer, A. et al. "The <i>Corynebacterium glutamicum</i> cglM gene encoding a S-cysteine in an MrC-deficient <i>Escherichia coli</i> strain," <i>Gene</i> , 203(2):95-101 (1997)
U14965	recA		Ankri, S. et al. "Mutations in the <i>Corynebacterium glutamicum</i> proline biosynthetic pathway: A natural bypass of the proA step," <i>J. Bacteriol.</i> , 178(15):4412-4419 (1996)
U31224	ppx		Ankri, S. et al. "Mutations in the <i>Corynebacterium glutamicum</i> glutamicum proline biosynthetic pathway: A natural bypass of the proA step," <i>J. Bacteriol.</i> , 178(15):4412-4419 (1996)
U31225	proC	L-proline NADP ⁺ oxidoreductase	Ankri, S. et al. "Mutations in the <i>Corynebacterium glutamicum</i> glutamicum proline biosynthetic pathway: A natural bypass of the proA step," <i>J. Bacteriol.</i> , 178(15):4412-4419 (1996)
U31230	obj; proB; unkdh	?gamma glutamyl kinase; similar to D. isomer specific 2-hydroxyacid dehydrogenases	Ankri, S. et al. "Mutations in the <i>Corynebacterium glutamicum</i> glutamicum proline biosynthetic pathway: A natural bypass of the proA step," <i>J. Bacteriol.</i> , 178(15):4412-4419 (1996)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
U31281	bioB	Biotin synthase	Serebriiskii, I.G., "Two new members of the bio B superfamily: Cloning, sequencing and expression of bio B genes of <i>Methylobacillus flagellatum</i> and <i>Corynebacterium glutamicum</i> ," <i>Gene</i> , 175:15-22 (1996)
U35023	thiR; accBC	Thiosulfate sulfurtransferase; acyl CoA carboxylase	Jager, W. et al. "A <i>Corynebacterium glutamicum</i> gene encoding a two-domain protein similar to biotin carboxylases and biotin-carboxyl-carrier proteins," <i>Arch Microbiol.</i> 166(2):76-82 (1996)
U43535	cml	Multidrug resistance protein	Jager, W. et al. "A <i>Corynebacterium glutamicum</i> gene conferring multidrug resistance in the heterologous host <i>Escherichia coli</i> ," <i>J Bacteriol.</i> 179(7):2449-2451 (1997)
U43536	cipB	Heat shock ATP-binding protein	
U53587	aphA-3	3'S'-aminoglycoside phosphotransferase	
U89648		<i>Corynebacterium glutamicum</i> unidentified sequence involved in histidine biosynthesis, partial sequence	Matsuji, K. et al. "Complete nucleotide and deduced amino acid sequences of the <i>Brevibacterium lactofermentum</i> tryptophan operon," <i>Nucleic Acids Res.</i> 14(24):10113-10114 (1986)
X04960	tppA; tppB; tppC; tppD; tppE; tppG; tppI	Tryptophan operon	Yeh, P. et al. "Nucleic sequence of the <i>lysA</i> gene of <i>Corynebacterium glutamicum</i> and possible mechanisms for modulation of its expression," <i>Mol Gen Genet.</i> 212(1):112-119 (1988)
X07363	lysA	DAP decarboxylase (meso-diaminopimelic decarboxylase, EC 4.1.1.20)	Eikmanns, B.J. et al. "The Phosphoenolpyruvate carboxylase gene of <i>Corynebacterium glutamicum</i> : Molecular cloning, nucleotide sequence, and expression," <i>Mol Gen Genet.</i> 218(2):330-339 (1989); Lepiniec, L. et al. "Sorghum Phosphoenolpyruvate carboxylase gene family: structure, function and molecular evolution," <i>Plant Mol Biol.</i> 21 (3):487-502 (1993)
X14234	EC 4.1.1.31	Phosphoenolpyruvate carboxylase	Von den Osten, C.H. et al. "Molecular cloning, nucleotide sequence and functional analysis of the <i>Corynebacterium glutamicum fda</i> gene: structural comparison of C. glutamicum fructose-1,6-biphosphate aldolase to class I and class II aldolases," <i>Mol Microbiol.</i>
X17313	fda	Fructose-bisphosphate aldolase	Bonnascie, S. et al. "Nucleic sequence of the <i>dapA</i> gene from <i>Corynebacterium glutamicum</i> ," <i>Nucleic Acids Res.</i> 18(2):6421 (1990)
X53393	dapA	1,2,3-dihydroxyproline synthetase (EC 4.2.1.52)	

GeneRank™ Accession No.	Gene Name	Gene Function	Reference
X54223		AttB-related site	Cianciotto, N. et al. "DNA sequence homology between att B-related sites of <i>Corynebacterium diphtheriae</i> , <i>Corynebacterium ulcerans</i> , <i>Corynebacterium glutamicum</i> , and the attP site of lambda corynephage," <i>fEMSS Microbiol.</i> , <i>Lett.</i> , 66:299-302 (1990).
X54740	argS; lysA	Arginyl-tRNA synthetase; Diaminopimelate decarboxylase	Marcel, T. et al. "Nucleotide sequence and organization of the upstream region of the <i>Corynebacterium glutamicum</i> lysA gene," <i>Mol Microbiol</i> , 4(11):1819-1830 (1990).
X55994	trpL; trpE	Putative leader peptide; anthranilate synthase component 1	Heery, D. M. et al. "Nucleotide sequence of the <i>Corynebacterium glutamicum</i> trpE gene," <i>Nucleic Acids Res.</i> , 18(23):7138 (1990).
X56037	thrC	Threonine synthase	Han, K. S. et al. "The molecular structure of the <i>Corynebacterium glutamicum</i> threonine synthase gene," <i>Mol Microbiol</i> , 4(10):1693-1702 (1990).
X56075	attB-related site	Attachment site	Cianciotto, N. et al. "DNA sequence homology between att B-related sites of <i>Corynebacterium diphtheriae</i> , <i>Corynebacterium ulcerans</i> , <i>Corynebacterium glutamicum</i> , and the attP site of lambda corynephage," <i>fEMSS Microbiol.</i> <i>Lett.</i> , 66:299-302 (1990).
X57226	lysC-alpha; lysC-beta; asd	Aspartokinase-alpha subunit; Aspartate beta semialdehyde dehydrogenase	Kalinowski, J. et al. "Genetic and biochemical analysis of the Aspartokinase from <i>Corynebacterium glutamicum</i> ," <i>Mol Microbiol</i> , 5(5):1197-1204 (1991); Kalinowski, J. et al. "Aspartokinase genes lysC alpha and lysC beta overlap and are adjacent to the aspartate beta-semialdehyde dehydrogenase gene and in <i>Corynebacterium glutamicum</i> ," <i>Mol Gen Genet</i> , 224(3):317-324 (1990).
X59403	gap; pgk; lpi	Glyceraldehyde-3-phosphate phosphoglycerate kinase, triosephosphate isomerase	Eikmanns, B. J. "Identification, sequence analysis, and expression of a <i>Corynebacterium glutamicum</i> gene cluster encoding the three glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, and triosephosphate isomerase," <i>J Bacteriol</i> , 174(19):6076-6086 (1992).
X59404	gdh	Glutamate dehydrogenase	Bormann, E. R. et al. "Molecular analysis of the <i>Corynebacterium glutamicum</i> gdh gene encoding glutamate dehydrogenase," <i>Mol Microbiol</i> , 6(1):317-326 (1992).
X60312	lysI	L-lysine permease	Seep-Feldhaus, A. J. et al. "Molecular analysis of the <i>Corynebacterium glutamicum</i> lysI gene involved in lysine uptake," <i>Mol Microbiol</i> , 5(12):2995-3005 (1991).

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X66078	cop1	PS1 protein	Joliff, G. et al. "Cloning and nucleotide sequence of the <i>csp1</i> gene encoding PS1, one of the two major secreted proteins of <i>Corynebacterium glutamicum</i> : The deduced N-terminal region of PS1 is similar to the Mycobacterium antigen 85 complex," <i>Mol. Microbiol.</i> , 6(16), 2349-2362 (1992)
X66112	gltI	Citrulline synthase	Eikmanns, B.J. et al. "Cloning sequence, expression and transcriptional analysis of the <i>Corynebacterium glutamicum gltA</i> gene encoding citrulline synthase," <i>Microbiol.</i> , 140:1817-1828 (1994)
X67737	dapB	Dihydrodipicolinate reductase	Peyrel, J.L. et al. "Characterization of the <i>cspB</i> gene encoding PS2, an ordered surface-layer protein in <i>Corynebacterium glutamicum</i> ," <i>Mol. Microbiol.</i> , 9(1):97-109 (1993)
X69103	csp2	Surface layer protein PS2	
X69104		IS3 related insertion element	Bonamy, C. et al. "Identification of IS1206, a <i>Corynebacterium glutamicum</i> IS3-related insertion sequence and phylogenetic analysis," <i>Mol. Microbiol.</i> , 14(3):571-581 (1994)
X70959	leuV	Isopropylmalate synthase	Patek, M. et al. "Leucine synthesis in <i>Corynebacterium glutamicum</i> : enzyme activities, structure of <i>leuA</i> , and effect of <i>leuA</i> inactivation on lysine synthesis," <i>Appl. Environ. Microbiol.</i> , 60(1):133-140 (1994)
X71489	icd	Isocitrate dehydrogenase (NADP ⁺)	Eikmanns, B.J. et al. "Cloning sequence analysis, expression, and inactivation of the <i>Corynebacterium glutamicum icd</i> gene encoding isocitrate dehydrogenase and biochemical characterization of the enzyme," <i>J. Bacteriol.</i> , 177(3):774-782 (1995)
X72855	GDHA	Glutamate dehydrogenase (NADP ⁺)	Heery, D.M. et al. "A sequence from a tryptophan-hypoxanthine-producing strain of <i>Corynebacterium glutamicum</i> encoding resistance to 5-methyltryptophan," <i>Biochem. Biophys. Res. Commun.</i> , 201(3):1255-1262 (1994)
X75083	mttA		Fitzpatrick, R. et al. "Construction and characterization of <i>recA</i> mutant strains of <i>Corynebacterium glutamicum</i> and <i>Brevibacterium lactosfermentum</i> ," <i>Appl. Microbiol. Biotechnol.</i> , 42(4):575-580 (1994)
X75084			
X75085	iccA		Reinschel, D.J. et al. "Characterization of the isocitrate lyase gene from <i>Corynebacterium glutamicum</i> and biochemical analysis of the enzyme," <i>J. Bacteriol.</i> , 176(12):3474-3483 (1994)
X75504	aceA; thiX	Partial isocitrate lyase;?	Ludwig, W. et al. "Phylogenetic relationships of bacteria based on comparative sequence analysis of elongation factor Tu and ATP-synthase beta-subunit genes," <i>Antonie Van Leeuwenhoek</i> , 64:285-305 (1993)
X76875		ATPase beta-subunit	

GenBank TM Accession No.	Gene Name	Gene Function	Reference
X77034	tuf	Elongation factor Tu	Ludwig, W. et al. "Phylogenetic relationships of bacteria based on comparative sequence analysis of elongation factor Tu and λ -TP-synthase beta-subunit genes," <i>Antonie Van Leeuwenhoek</i> , 64:283-305 (1993)
X77384	iceA		Billman-Jaenicke, H. "Nucleotide sequence of a recA gene from <i>Corynebacterium glutamicum</i> ," <i>DNA Seq.</i> , 4(6):403-404 (1994)
X78491	aceB	Malate synthase	Reinscheid, D.J. et al. "Malate synthase from <i>Corynebacterium glutamicum</i> : plasmid question encoding phosphotransacetylase: sequence analysis," <i>Microbiology</i> , 140:3099-3108 (1994)
X80629	16S rDNA	16S ribosomal RNA	Rainey, F.A. et al. "Phylogenetic analysis of the genus <i>Rhodococcus</i> and <i>Neocardia</i> and evidence for the evolutionary origin of the genus <i>Neocardia</i> from within the radiation of <i>Rhodococcus</i> species," <i>Microbiol.</i> , 141:523-528 (1995)
X81191	gluA; gluB; gluC; gluD	Glutamate uptake system	Kronemeyer, W. et al. "Structure of the GluABCD cluster encoding the glutamate uptake system of <i>Corynebacterium glutamicum</i> ," <i>J Bacteriol.</i> , 177(5):1152-1158 (1995)
X81379	dapE	Succinyldiaminopimelate desuccinylase	Wehmann, A. et al. "Analysis of different DNA fragments of <i>Corynebacterium glutamicum</i> complementing dapE of <i>Escherichia coli</i> ," <i>Microbiology</i> , 140:3349-3356 (1994)
X82061	16S rDNA	16S ribosomal RNA	Ruimy, R. et al. "Phylogeny of the genus <i>Corynebacterium</i> deduced from analyses of small subunit ribosomal DNA sequences," <i>Int. J. Syst. Bacteriol.</i> , 45(4):740-746 (1995)
X82928	asd; hisC	Aspartate-semialdehyde dehydrogenase; ?	Sechijiski, I. et al. "Multicopy suppression by asd gene and osmotic stress-dependent complementation by heterologous proA in proA mutants," <i>J Bacteriol.</i> , 177(24):7255-7260 (1995)
X82929	proA	Gamma-glutamyl phosphate reductase	Sechijiski, I. et al. "Multicopy suppression by asd gene and osmotic stress-dependent complementation by heterologous proA in proA mutants," <i>J Bacteriol.</i> , 177(24):7255-7260 (1995)
X84257	16S rDNA	16S ribosomal RNA	Pascual, C. et al. "Phylogenetic analysis of the genus <i>Corynebacterium</i> based on 16S rRNA gene sequences," <i>Int. J. Syst. Bacteriol.</i> , 45(4):724-728 (1995)
X85965	aroP; dapE	Aromatic amino acid permease; ?	Wehmann, A. et al. "Functional analysis of sequences adjacent to dapE of <i>Corynebacterium glutamicum</i> proline reveals the presence of aop, which encodes the aromatic amino acid transporter," <i>J Bacteriol.</i> , 177(20):5991-5993 (1995)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X86157	aigB; aigC; aigD; aigF; aigJ	Acetylglutamate kinase; N-acetyl-gamma-glutamyl-phosphopeptide reduclease; acetylornithine aminotransferase; ornithine carbamoyltransferase; glutamate N-acetyltransferase	Sakanyan, V. et al. "Genes and enzymes of the acetyl cycle of arginine biosynthesis in <i>Corynebacterium glutamicum</i> : enzyme evolution in the early steps of the arginine pathway." <i>Microbiology</i> , 142:99-108 (1996)
X89084	pta; ackA	Phosphate acetyltransferase, acetate kinase	Reinschid, D.J. et al. "Cloning, sequence analysis, expression and inactivation of the <i>Corynebacterium glutamicum</i> pta-ack operon encoding phosphotransacetylase and acetate kinase." <i>Microbiology</i> , 145:503-513 (1999)
X89650	attB	Attachment site	Le Marre, C. et al. "Genetic characterization of site-specific integration functions of phi A112 infecting 'Arthrobacter aurus C70'." <i>J. Bacteriol.</i> 178(7):1996-2004 (1996)
X90356		Promoter fragment F1	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif." <i>Microbiology</i> , 142:1297-1309 (1996)
X90357		Promoter fragment F2	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif." <i>Microbiology</i> , 142:1297-1309 (1996)
X90358		Promoter fragment F10	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif." <i>Microbiology</i> , 142:1297-1309 (1996)
X90359		Promoter fragment F13	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif." <i>Microbiology</i> , 142:1297-1309 (1996)
X90360		Promoter fragment F22	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif." <i>Microbiology</i> , 142:1297-1309 (1996)
X90361		Promoter fragment F34	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif." <i>Microbiology</i> , 142:1297-1309 (1996)
X90362		Promoter fragment F37	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif." <i>Microbiology</i> , 142:1297-1309 (1996)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X90363	Promoter fragment F43	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif," <i>Microbiology</i> , 142:1297-1309 (1996)	
X90364	Promoter fragment F64	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif," <i>Microbiology</i> , 142:1297-1309 (1996)	
X90365	Promoter fragment F75	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif," <i>Microbiology</i> , 142:1297-1309 (1996)	
X90366	Promoter fragment PF101	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif," <i>Microbiology</i> , 142:1297-1309 (1996)	
X90367	Promoter fragment PF104	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif," <i>Microbiology</i> , 142:1297-1309 (1996)	
X90368	Promoter fragment PF109	Patek, M. et al. "Promoters from <i>Corynebacterium glutamicum</i> : cloning, molecular analysis and search for a consensus motif," <i>Microbiology</i> , 142:1297-1309 (1996)	
X93513	amt	Ammonium transport system Siewe, R. M. et al. "Functional and genetic characterization of the (methyl) ammonium uptake carrier of <i>Corynebacterium glutamicum</i> ," <i>J. Biol. Chem.</i> , 271(10):5598-5603 (1996)	
X93514	bclP	Glycine betaine transport system Patek, M. et al. "Isolation, characterization, and expression of the <i>Corynebacterium glutamicum</i> bclP gene, encoding the transport system for the compatible solute glycine betaine," <i>J. Bacteriol.</i> , 178(17):5229-5234 (1996)	
X95649	orf4	dapA·ORF4 operon of <i>Corynebacterium glutamicum</i> , encoding two enzymes involved in L-lysine synthesis: "Biotechnol. Lett.", 19:1113-1117 (1997)	
X96471	LysE; LysG	Lysine exporter protein, Lysine export regulator protein Vrijic, M. et al. "A new type of transporter with a new type of cellular function: L-lysine export from <i>Corynebacterium glutamicum</i> ," <i>Microbiol.</i> , 22(5):815-826 (1996)	

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X96580	panB, panC; xylB	3-methyl-2-oxobutanoate hydroxymethyltransferase; pantoate-beta-alanine ligase; xylulokinase	Sahn, H. et al. "D-pantothenate synthesis in <i>Corynebacterium glutamicum</i> and use of panBC and genes encoding L-valine synthesis for D-pantothenate overproduction," <i>Appl Environ Microbiol</i> , 65(5), 1973-1979 (1999)
X96962		Insertion sequence IS207 and transposase	Ramos, A. et al. "Cloning, sequencing and expression of the gene encoding elongation factor P in the amino-acid producer <i>Brevibacterium lactofermentum</i> (Corynebacterium glutamicum ATCC 13869)," <i>Gene</i> , 198: 217-222 (1997)
X99289		Elongation factor P	Malcos, L.M. et al. "Nucleotide sequence of the homoserine kinase (thiB) gene of the <i>Brevibacterium lactofermentum</i> ," <i>Nucleic Acids Res.</i> , 15(9):3922 (1987)
Y00140	thiB	Homoserine kinase	Ishino, S. et al. "Nucleotide sequence of the meso-diaminopimelate D-dehydrogenase gene from <i>Corynebacterium glutamicum</i> ," <i>Nucleic Acids Res.</i> , 15(9):3917 (1987)
Y00151	ddh	Meso-diaminopimelate D-dehydrogenase (EC 1.4.1.16)	Malcos, L.M. et al. "Nucleotide sequence of the <i>Brevibacterium lactofermentum</i> (thiA) gene of the <i>Brevibacterium lactofermentum</i> ," <i>Nucleic Acids Res.</i> , 15(24):10598 (1987)
Y00476	thiA	Homoserine dehydrogenase	Peoples, O.P. et al. "Nucleotide sequence and fine structural analysis of the <i>Corynebacterium glutamicum</i> hom-thiB operon," <i>Mol Microbiol</i> , 2(1):63-72 (1988)
Y00546	hom, thiB	Homoserine dehydrogenase; homoserine kinase	Honrubia, M.P. et al. "Identification, characterization, and chromosomal organization of the <i>flsZ</i> gene from <i>Brevibacterium lactofermentum</i> ," <i>Mol Gen Genet</i> , 259(1):97-104 (1998)
Y08964	murC, ftsQ/divD; ftsZ	UPD-N-acetylumuramate-alanine ligase, division initiation protein or cell division protein; cell division protein	Pcler, H. et al. "Isolation of the <i>pulP</i> gene of <i>Corynebacterium glutamicum</i> and characterization of a low-affinity uptake system for compatible solutes," <i>Arch Microbiol.</i> , 168(2): 143-151 (1997)
Y09163	pulP	High affinity proline transport system	Peters-Wendisch, P.G. et al. "Pyruvate carboxylase from <i>Corynebacterium glutamicum</i> : characterization, expression and inactivation of the <i>pyc</i> gene," <i>Microbiology</i> , 144:915-927 (1998)
Y09548	pyc	Pyruvate carboxylase	Palick, M. et al. "Analysis of the <i>leuB</i> gene from <i>Corynebacterium glutamicum</i> ," <i>Appl Microbiol Biotechnol</i> , 50(1):42-47 (1998)
Y09578	leuB	3-isopropylmalate dehydrogenase	Moreau, S. et al. "Site-specific integration of corynephage Phi-16: The construction of an integration vector," <i>Microbiol</i> , 145:539-548 (1999)
Y12472		Attachment site bacteriophage Phi-16	

GenBank™ Accession No.	Gene Name	Gene Function	Reference
Y12537	proP	Proline/ectoine uptake system protein	Peter, H. et al. "Corynebacterium glutamicum is equipped with four secondary carriers for compatible solutes. Identification, sequencing, and characterization of the proline/ectoine uptake system, ProP, and the ectoine/proline/glycine betaine carrier, EcpP." <i>J. Bacteriol.</i> , 180(22):6005-6012 (1998)
Y13221	glnA	Glutamine synthetase 1	Jakoby, M. et al. "Isolation of Corynebacterium glutamicum glnA gene encoding glutamine synthetase 1." <i>FEMS Microbiol Lett.</i> , 154(1):81-88 (1997)
Y16642	ldp	Dihydrodipicolamide dehydrogenase	Moreau, S. et al. "Analysis of the integration functions of & phi;304L. An integrase module among corynephages." <i>Virology</i> , 253(1):150-159 (1999)
Y18059	lysA	Attachment site Corynephage 304L	Oguiza, J. A. et al. "A gene encoding arginyl-tRNA synthetase is located in the upstream region of the lysA gene in Brevibacterium lactofermentum. Regulation of argS-lysA cluster expression by arginine." <i>J. Bacteriol.</i> , 175(22):7356-7362 (1993)
221501	argS; lysA	Arginyl-tRNA synthetase; diaminopimelate decarboxylase (partial)	Pisabarro, A. et al. "A cluster of three genes (dapA, orf2, and dapB) of Brevibacterium lactofermentum encodes dihydrotropicoline reductase, and a third polypeptide of unknown function." <i>J. Bacteriol.</i> , 175(9):2743-2749 (1993)
221502	dapP; dapB	Dihydrotropicoline synthase; dihydrotropicoline reductase	Mahmoudies, M. et al. "Analysis and expression of the thrC gene of the encoded threonine synthase." <i>Appl. Environ. Microbiol.</i> , 60(7):2209-2219 (1994)
229563	thrC	Threonine synthase	Oguiza, J. A. et al. "Multiple sigma factor genes in Brevibacterium lactofermentum. Characterization of sigA and sigB." <i>J. Bacteriol.</i> , 178(2):550-553 (1996)
246753	16S rDNA	Gene for 16S ribosomal RNA	Oguiza, J. A. et al. "The galE gene encoding the UDP-galactose 4-epimerase of Brevibacterium lactofermentum is coupled transcriptionally to the dmdR gene." <i>Gene</i> , 177:103-107 (1996)
249822	sigA	SigA sigma factor	Oguiza, J. A. et al. "Multiple sigma factor genes in Brevibacterium lactofermentum: Characterization of sigA and sigB." <i>J. Bacteriol.</i> , 178(2):550-553 (1996)
249823	galE; dmdR	Catalytic activity UDP-galactose 4-epimerase; diphtheria toxin regulatory protein	Corcilia, A. et al. "Cloning and characterization of an IS-like element present in the genome of Brevibacterium lactofermentum ATCC 13869." <i>Gene</i> , 170(1):91-94 (1996)
249824	orf1; sigB	?; SigB sigma factor	
266334		Transposase	

A sequence for this gene was published in the indicated reference. However, the sequence obtained by the inventors of the present application is significantly longer than the published version. It is believed that the published version relied on an incorrect start codon, and thus represents only a fragment of the actual coding region.

TABLE 3: *Corynebacterium* and *Brevibacterium* Strains Which May be Used in the Practice of Medicine

<i>Brevibacterium</i>	<i>flavum</i>			B11478
<i>Brevibacterium</i>	<i>flavum</i>	21127		
<i>Brevibacterium</i>	<i>flavum</i>		B11474	
<i>Brevibacterium</i>	<i>halii</i>	15527		
<i>Brevibacterium</i>	<i>keelingi</i>	21004		
<i>Brevibacterium</i>	<i>ketoglutamicum</i>	21089		
<i>Brevibacterium</i>	<i>ketosoductum</i>	21914		
<i>Brevibacterium</i>	<i>lactofermentum</i>		70	
<i>Brevibacterium</i>	<i>lactofermentum</i>			74
<i>Brevibacterium</i>	<i>lactofermentum</i>			77
<i>Brevibacterium</i>	<i>lactofermentum</i>	21798		
<i>Brevibacterium</i>	<i>lactofermentum</i>	21799		
<i>Brevibacterium</i>	<i>lactofermentum</i>	21800		
<i>Brevibacterium</i>	<i>lactofermentum</i>	21801	B11470	
<i>Brevibacterium</i>	<i>lactofermentum</i>			B11471
<i>Brevibacterium</i>	<i>lactofermentum</i>	21086		
<i>Brevibacterium</i>	<i>lactofermentum</i>	21420		
<i>Brevibacterium</i>	<i>lactofermentum</i>	21086		
<i>Brevibacterium</i>	<i>lactofermentum</i>	31269		
<i>Brevibacterium</i>	<i>lactic</i>	9174		
<i>Brevibacterium</i>	<i>linens</i>			19391
<i>Brevibacterium</i>	<i>linens</i>			8377
<i>Brevibacterium</i>	<i>parafimoliticum</i>			11160
<i>Brevibacterium</i>	<i>spic.</i>			717.73
<i>Brevibacterium</i>	<i>spic.</i>			717.73
<i>Brevibacterium</i>	<i>spic.</i>	14604		
<i>Brevibacterium</i>	<i>spic.</i>	21860		
<i>Brevibacterium</i>	<i>spic.</i>	21864		
<i>Brevibacterium</i>	<i>spic.</i>	21865		
<i>Brevibacterium</i>	<i>spic.</i>	21866		
<i>Brevibacterium</i>	<i>spic.</i>	19240		

<i>Corynebacterium</i>	<i>acetoxidophilum</i>	21476	
<i>Corynebacterium</i>	<i>acetoacidophilum</i>	13870	
<i>Corynebacterium</i>	<i>acetoglutamicum</i>		B11473
<i>Corynebacterium</i>	<i>acetoglutamicum</i>		B11475
<i>Corynebacterium</i>	<i>acetoglutamicum</i>	15806	
<i>Corynebacterium</i>	<i>acetoglutamicum</i>	21491	
<i>Corynebacterium</i>	<i>acetoglutamicum</i>	31270	
<i>Corynebacterium</i>	<i>acetophilum</i>		B3671
<i>Corynebacterium</i>	<i>ammoniogenes</i>	6872	
<i>Corynebacterium</i>	<i>ammoniogenes</i>	15511	
<i>Corynebacterium</i>	<i>fujikense</i>	21496	
<i>Corynebacterium</i>	<i>glutamicum</i>	14067	
<i>Corynebacterium</i>	<i>glutamicum</i>	39137	
<i>Corynebacterium</i>	<i>glutamicum</i>	21254	
<i>Corynebacterium</i>	<i>glutamicum</i>	21255	
<i>Corynebacterium</i>	<i>glutamicum</i>	31830	
<i>Corynebacterium</i>	<i>glutamicum</i>	13032	
<i>Corynebacterium</i>	<i>glutamicum</i>	14305	
<i>Corynebacterium</i>	<i>glutamicum</i>	15455	
<i>Corynebacterium</i>	<i>glutamicum</i>	13058	
<i>Corynebacterium</i>	<i>glutamicum</i>	13059	
<i>Corynebacterium</i>	<i>glutamicum</i>	13060	
<i>Corynebacterium</i>	<i>glutamicum</i>	21492	
<i>Corynebacterium</i>	<i>glutamicum</i>	21513	
<i>Corynebacterium</i>	<i>glutamicum</i>	21526	
<i>Corynebacterium</i>	<i>glutamicum</i>	21543	
<i>Corynebacterium</i>	<i>glutamicum</i>	13287	
<i>Corynebacterium</i>	<i>glutamicum</i>	21851	
<i>Corynebacterium</i>	<i>glutamicum</i>	21253	
<i>Corynebacterium</i>	<i>glutamicum</i>	21514	
<i>Corynebacterium</i>	<i>glutamicum</i>	21516	
<i>Corynebacterium</i>	<i>glutamicum</i>	21299	

<i>Corynebacterium</i>	<i>Glutamicum</i>	21300	
<i>Corynebacterium</i>	<i>Glutamicum</i>	39684	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21488	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21649	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21650	
<i>Corynebacterium</i>	<i>Glutamicum</i>	19223	
<i>Corynebacterium</i>	<i>Glutamicum</i>	13869	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21157	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21158	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21159	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21355	
<i>Corynebacterium</i>	<i>Glutamicum</i>	31808	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21674	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21562	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21563	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21564	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21565	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21566	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21567	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21568	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21569	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21570	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21571	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21572	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21573	
<i>Corynebacterium</i>	<i>Glutamicum</i>	21579	
<i>Corynebacterium</i>	<i>Glutamicum</i>	19049	
<i>Corynebacterium</i>	<i>Glutamicum</i>	19050	
<i>Corynebacterium</i>	<i>Glutamicum</i>	19051	
<i>Corynebacterium</i>	<i>Glutamicum</i>	19052	
<i>Corynebacterium</i>	<i>Glutamicum</i>	19053	
<i>Corynebacterium</i>	<i>Glutamicum</i>	19054	

<i>Corynebacterium</i>	<i>glutamicum</i>	19055
<i>Corynebacterium</i>	<i>glutamicum</i>	19056
<i>Corynebacterium</i>	<i>glutamicum</i>	19057
<i>Corynebacterium</i>	<i>glutamicum</i>	19058
<i>Corynebacterium</i>	<i>glutamicum</i>	19059
<i>Corynebacterium</i>	<i>glutamicum</i>	19060
<i>Corynebacterium</i>	<i>glutamicum</i>	19185
<i>Corynebacterium</i>	<i>glutamicum</i>	19286
<i>Corynebacterium</i>	<i>glutamicum</i>	21515
<i>Corynebacterium</i>	<i>glutamicum</i>	21527
<i>Corynebacterium</i>	<i>glutamicum</i>	21544
<i>Corynebacterium</i>	<i>glutamicum</i>	21492
<i>Corynebacterium</i>	<i>glutamicum</i>	B8183
<i>Corynebacterium</i>	<i>glutamicum</i>	B8182
<i>Corynebacterium</i>	<i>glutamicum</i>	B12416
<i>Corynebacterium</i>	<i>glutamicum</i>	B12417
<i>Corynebacterium</i>	<i>glutamicum</i>	B12418
<i>Corynebacterium</i>	<i>glutamicum</i>	B11476
<i>Corynebacterium</i>	<i>glutamicum</i>	21608
<i>Corynebacterium</i>	<i>glutamicum</i>	1973
<i>Corynebacterium</i>	<i>hium</i>	11594
<i>Corynebacterium</i>	<i>nitrilophilus</i>	21419
<i>Corynebacterium</i>	<i>spec.</i>	P4445
<i>Corynebacterium</i>	<i>spec.</i>	P4446
<i>Corynebacterium</i>	<i>spec.</i>	31088
<i>Corynebacterium</i>	<i>spec.</i>	31089
<i>Corynebacterium</i>	<i>spec.</i>	31090
<i>Corynebacterium</i>	<i>spec.</i>	15954
<i>Corynebacterium</i>	<i>spec.</i>	21857
<i>Corynebacterium</i>	<i>spec.</i>	21862
<i>Corynebacterium</i>	<i>spec.</i>	21863
<i>Corynebacterium</i>	<i>spec.</i>	20145

ATCC: American Type Culture Collection, Rockville, MD, USA

FERM: Fermentation Research Institute, Chiba, Japan

NRRL: ARS Culture Collection, Northern Regional Research Laboratory, Peoria, IL, USA

CECT: Colección Española de Cultivos Tipo, Valencia, Spain

NCIMB: National Collection of Industrial and Marine Bacteria Ltd., Aberdeen, UK

CBS: Centraalbureau voor Schimmelcultures, Baarn, NL

NCTC: National Collection of Type Cultures, London, UK

DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany

For reference see Sugawara, H et al. (1993) World directory of collections of cultures of microorganisms: Bacteria, fungi and yeasts (4th edn), World federation for culture collections world data center on microorganisms, Saitama, Japan.

Appendix A & B

>>RXA01715-amino acid sequence
(1-696, translated) 232 residues

VSELDIQLN KIQRYSQAWAV FRAIPGALDD DRTEVTDQAA KFFADLEAEG KVTVRGIYNA SGLRADADYM
IWWHAEEFED IQKAFADFRR TTILGQVSEV FWIGNALHRP SEFNKAHLPS FIMGEEAKDW ITVYPFVRSY
DWYIMEPLKR SRILREHGQA AVEFPDVRAN TVPAFALGDY EWVLAFAADE LHRIVDLMHK MRYTEARLHV
REELPFISGQ RVDIADLIKV LP

>RXA01715-nucleotide sequence A: upstream

ACATGTTGGAACATGCCGGCAGAGCCGACACTACGATTCAATTGCTAAAGGGTCTGGCCACTGACACTGGCAAA
GATCCACGAAAGGAAGTTACCCCT

>RXA01715-nucleotide sequence B: coding region

GTGAGCGAGCTCGATATTAAACAGCTAACAAACTGCAGCGCTACTCTCAGTGGCGGTGTTCCGTGCTATTCCCTGG
AGCGCTCGATGATGATCGCACAGAAGTCAGTACCGACCAAGCAGCCAAGTTCTTGCCGACCTTGAAAGCAGAAGGCAAAG
TCACTGTCCGTGGCATTACAACGCCCTCCGGCTGCGCGACGCTGACTACATGATCTGGTGGCACCGCAGAAGAA
TTCGAAGACATTAGAAGGCCTTCGCTGATTTCGCGCACCACCAATTGGGTCAAGGTTCTGAGGTCTTCTGGAT
CGGAAACGCTCTCCACCGTCCATCTGAGTTCAACAAGGCTCACTTGCCCTCATTCATCATGGGTGAAGAAGCAAAGG
ACTGGATCACTGTTACCCGTTCGTGCAGCTACGACTGGTACATCATGGGACCCCTTGAAAGCGTTCCCGCATTCTC
CGCGAGCACGGACAAGCTGCTGTGGATTCCAGATGTTGCGCAACACTGTGCGGGCTTCGCACACTGGTGACTA
CGAATGGGTGCTGGCTTCGAGGCTGATGAGTTGCACCGCATTGTCGATTGTCACAGATGCGTTACACCGAGG
CTCGCCTCCACGTCCGTGAGGAGCTGCCATTATTCTGGACAGCGCGTCGACATTGCAAGATCTGATTAAAGGTTCTT
CCT

>RXA01715-nucleotide sequence C: downstream

TAAAAGCTGCTTTCTAAACGAT

Appendix A & B

>>RXA01709-amino acid sequence
(1-330, translated) 110 residues

MRS GVDMILNETG GEKMLAQADL VITGEGRIDA QTLSGKAPTG IAKRARAKGI PVLAVCGQSL
LGPAISNELF EDIYSFTDFE SDINECIRNP LPILEGIGFN IAKHHL

>RXA01709-nucleotide sequence A: upstream
TTGTTGTCTGCAGGG

>RXA01709-nucleotide sequence B: coding region

ATGCGCTCCGGCGTGGACATGATTCTTAATGAAACCGGGGTGAAAAGATGCTTGCACAGGCAGATTAGTCATCAC
TGGAGAAGGACGCATTGATGCACAGACCTCAGCGGAAAGCTCCTACTGGAATGCCAACGGGCACGTGCGAAAG
GAATTCCAGTACTGGCGTTGTGGGAGAGCCTATTGGGTCCAGCAATCTCAAATGAGCTATTGAAGACATCTAC

AGCTTTACCGATTCGAATCTGACATCAATGAATGCATTGAAACCCGCTCCAATTTGGAAGGTATCGGTTTAA

CATGCCAACATCATCTGAGT

>RXA01709-nucleotide sequence C: downstream

TAGCGATATTCAGCAAACCGAT

Appendix A & B

>>RXA01662-amino acid sequence
(1-543, translated) 181 residues

VLDKWVNRAD LAESAINERH SARVWGLPRT NLGFVAWPSN AKEKLFIFHWH YWWQAHYLD C LVDAARRRTT
KARRDRIRDT IRGISVRNVG KLTSNRYYDD KAWLALALGR AGKVRKVRTP KSLPSLEQNI VDGIDS LTGV
LPWRFGETFY NVPNSNGPAAI MMARTDRLDE AMKIPIGFLT T

>RXA01662-nucleotide sequence A: upstream

AAGCCTGGTAATTTATACCTAGATCGTTAGACTTCGTATACCTTGGGTGTCGTATTAGTTGACAACACCT
GCATTGGAGCGAAGAAAACACTCA

>RXA01662-nucleotide sequence B: coding region

GTGTTAGACAAATGGGTAAACAGAGCTGACCTAGCGGAATCCGCCATCAACGAAAGGCATTCCGCGAGGGTTTGGGG
TCTGCCCTCGAACAAATCTGGGTTTGTGGCATGCCATCCAACGCCAAGAAAAACTGTTATCCACTGGCACTACT
GGTGGCAAGCGCATTATCTAGACTGCCCTGGTGGATGCTGCTCGACGCCACAAAGGCCGTCGCGACCGCATT
AGGGACACCATCCCGGCATTCGGTGCACATGTGGCAAGCTGACCTCGAACATCGTTATTACGACGACAAAGCTTG
GCTGCCCTTGCTTGGCGTGCACCGAACCAAAATCATTGCCCTGTTGGAACAAA
ACATCGTCGATGGCATTGATTCCCTACTGGTGTGCTGCCGTGGCGAACCTCTACAACGTTCCCTCC
AACGGTCCTGCTGCGATCATGATGGCCCGCACCGACCCTTGGACGAGGCTATGAAAATACCGATTGGATTGGAC
AACC

>RXA01662-nucleotide sequence C: downstream

TGATCGATGGCGACGGCCTTGTG

Appendix A & B

>>RXA01622-amino acid sequence
(1-603, translated) 201 residues

MSDFYADRLF NAMERNEVAP GMLLVAAPDM ASEDFERSIV LIEHSPATT FGVNIISSRSD VAVANVLPEW
VDLTSKPQAL YIGGPLSQQA VVGLGVTKPG VDIENSTSFN KLANRLVHD LRSAPEDVAD DLEGMRFFAG
YAEWAPQLN EEEIEQGDWFV TPALPSDIIA PGRVDIWGDV MRRQAMPLPL YSTFPSPDPSD N

>RXA01622-nucleotide sequence A: upstream

AAGCGTGGCGTTTGAGGAGCTTCGTTGGAGCGCGTCCTGGATCGTAAGTTGCCATCGCAGAGCTGAA
GAGCTGGTGGGAAGGAGAAAACA

>RXA01622-nucleotide sequence B: coding region

ATGAGTGATTTTATGCCGACAGGTTGTTAACCGATGGAGCGCAATGAGGTAGCTCCAGGCATGTTGGTGGC
TGGCCCGATATGGCGTCGGAGGATTTGAGCGCAGCATCGTGTGATCATCGAGCATTCTCTGCCACCACTTTG
GTGTGAACATTCTTCACGTTCCGATGTTGCTGAGGCAATGTTGGCTGAGTGGGTGGACCTCACCTCGAAGCCA
CAGGCACGTGATCATCGGTGGGCCGTTGAGCCAGCAGGCTGTTGGGTTGGCGTGACCAAGCCGGCGTGGATAT
TGAAAATTCCACCAAGCTCAACAAGCTCGCCAACCGCCTGGTGCACGTGGATCTCGTTCTGCACCTGAAGATGTGG
CTGATGATCTTGAGGGCATGCGCTTTTGCGGGCTACGCGGAGTGGGCTCCGGCCAGCTCAACGAGGAAATTGAG
CAGGGTGATTGGTTCGTCACACCTGCCGTTGGACATTATCGCGCCGGCCGCGTCAATTGGGGCGACGT
GATGCGTCGACAAGCAATGCCCTTGCGTTGTATTCCACGTTCCGACCCCTCAGATAAT

>RXA01622-nucleotide sequence C: downstream

TAGATGAGTTCCGAAAATTAAA

Appendix A & B

>>RXA01600-amino acid sequence
(1-669, translated) 223 residues

MVSKMHIPGT HEFTVTDTEL LLESPILGVR RDSLIMPFGS TARREVVEHF GAVAVVAFDG ENIAMVKQYR
RSVGDSLWEL PAGLLDIADE DELTGAQREL MEEAGLEASE WSVLTDLITS PGFCDEAVRV FLARGLTKVE
RPKVMGDEEA DMINQWVPLH EAVGMVFSQ LVNSIAIAGV MAADAVIAGR ASARAVTAPF TYRPTALAQR
RKAHGIVPDM KKL

>RXA01600-nucleotide sequence A: upstream

TGAGTACAAATCTCGTCAACCCATGCTCATCCACTGTTTACGGCTGGTGAAGACCGCTTGGAGCTGCGTGTCC
ACCCCTAGATCTACAATGTGATC

>RXA01600-nucleotide sequence B: coding region

ATGGTTTCGAAGATGCACATCCCGTACCCATGAGTTCACGGTGACAGATACTGAACGTGTTAGAGTCCCCAAT
TTTGGCGGTTCGTCGAGATTCAATTGATCATGCCGGTGGTCCACTGCCCGCCGTGAAGTGGTTGAACACTTTGGGG
CGGTCGAGTGGTTGCCCTTGATGGTGAACATTGCGATGGTCAAGCAGTACCGTCGCAGCGTGGGGGATTCCCTG
TGGGAGCTGCCTGCAGGTTGGATATTGCTGATGAGGATGAACTCACGGGCGCGCAGCGCAGCTCATGGAGGA
GGCTGGTTGGAGGCCAGTGACTGGTCCGTGCTACTGATTGATTACCTCGCCGGTTCTGCATGAAGCGGTGC
GTGTCTTCTAGCCCGAGGCCTCACAAAGGTTGAGCGCCGAAGGTTATGGCGATGAAGAACGGACATGATTAAC
CAGTGGTTCCGCTACATGAGGCAGTGGGAATGGTGTGTTAGTGGCCAGTTGGTTAACCTCCATTGCCATTGCGGGTGT
CATGGCTGCTGATGCTGTGATTGGGGTCTGCCGTGCCGTGCCGTACCGCGCCGTTACCTATGCCCTACGG
CGTTGGCGCAGCGTCGAAAAGCGCACGGCATTGTCCTGACATGAAAAAAACTA

>RXA01600-nucleotide sequence C: downstream

TGAAGGCTCGCGTTTAGCGAAA

Claims

1. An isolated nucleic acid molecule from *Corynebacterium glutamicum* encoding an MCP protein, or a portion thereof.
- 5 2. The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes an MCP protein involved in fine chemical production.
- 10 3. An isolated *Corynebacterium glutamicum* nucleic acid molecule selected from the group consisting of those sequences set forth in Appendix A, or a portion thereof.
4. An isolated nucleic acid molecule which encodes a polypeptide sequence selected from the group consisting of those sequences set forth in Appendix B.
- 15 5. An isolated nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide selected from the group of amino acid sequences consisting of those sequences set forth in Appendix B.
- 20 6. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 50% homologous to a nucleotide sequence selected from the group consisting of those sequences set forth in Appendix A, or a portion thereof.
7. An isolated nucleic acid molecule comprising a fragment of at least 15 nucleotides of a nucleic acid comprising a nucleotide sequence selected from the group consisting of those sequences set forth in Appendix A.
- 25 8. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of any one of claims 1-7 under stringent conditions.
- 30 9. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1-8 or a portion thereof and a nucleotide sequence encoding a heterologous polypeptide.
10. A vector comprising the nucleic acid molecule of any one of claims 1-9.
- 35 11. The vector of claim 10, which is an expression vector.
12. A host cell transfected with the expression vector of claim 11.
- 40 13. The host cell of claim 12, wherein said cell is a microorganism.
14. The host cell of claim 13, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.
- 45 15. The host cell of claim 12, wherein the expression of said nucleic acid molecule results in the modulation in production of a fine chemical from said cell.

16. The host cell of claim 15, wherein said fine chemical is selected from the group consisting of: organic acids, proteinogenic and nonproteinogenic amino acids, purine and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, and enzymes.

5 17. A method of producing a polypeptide comprising culturing the host cell of claim 12 in an appropriate culture medium to, thereby, produce the polypeptide.

10 18. An isolated MCP polypeptide from *Corynebacterium glutamicum*, or a portion thereof.

15 19. The polypeptide of claim 18, wherein said polypeptide is involved in fine chemical production.

20 20. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth in Appendix B.

25 21. An isolated polypeptide comprising a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth in Appendix B, or a portion thereof.

22. The isolated polypeptide of any of claims 18-21, further comprising heterologous amino acid sequences.

25 23. An isolated polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 50% homologous to a nucleic acid selected from the group consisting of those sequences set forth in Appendix A.

30 24. An isolated polypeptide comprising an amino acid sequence which is at least 50% homologous to an amino acid sequence selected from the group consisting of those sequences set forth in Appendix B.

35 25. A method for producing a fine chemical, comprising culturing a cell containing a vector of claim 12 such that the fine chemical is produced.

26. The method of claim 25, wherein said method further comprises the step of recovering the fine chemical from said culture.

40 27. The method of claim 25, wherein said method further comprises the step of transfecting said cell with the vector of claim 11 to result in a cell containing said vector.

45 28. The method of claim 25, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.

29. The method of claim 25, wherein said cell is selected from the group consisting of: *Coryncbacterium glutamicum*, *Corynebacterium herculis*, *Corynebacterium lilium*, *Corynebacterium acetoacidophilum*, *Corynebacterium acetoglutamicum*.

Corynebacterium acetophilum. Corynebacterium ammoniogenes. Corynebacterium fujikense. Corynebacterium nitrilophilus. Brevibacterium ammoniagenes. Brevibacterium butanicum. Brevibacterium divaricatum. Brevibacterium flavum. Brevibacterium healii. Brevibacterium ketoglutamicum. Brevibacterium ketosoreductum. Brevibacterium lactofermentum. Brevibacterium linens. Brevibacterium paraffinolyticum. and those strains set forth in Table 3.

5 30. The method of claim 25, wherein expression of the nucleic acid molecule from said vector results in modulation of production of said fine chemical.

10 31. The method of claim 25, wherein said fine chemical is selected from the group consisting of: organic acids, proteinogenic and nonproteinogenic amino acids, purine and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, and enzymes.

15 32. The method of claim 25, wherein said fine chemical is an amino acid.

20 33. The method of claim 32, wherein said amino acid is drawn from the group consisting of: lysine, glutamate, glutamine, alanine, aspartate, glycine, serine, threonine, methionine, cysteine, valine, leucine, isoleucine, arginine, proline, histidine, tyrosine, phenylalanine, and tryptophan.

25 34. A method for producing a fine chemical, comprising culturing a cell whose genomic DNA has been altered by the inclusion of a nucleic acid molecule of any one of claims 1-9.

CORYNEBACTERIUM GLUTAMICUM GENES ENCODING NOVEL PROTEINS

Abstract of the Disclosure

5

Isolated nucleic acid molecules, designated MCP nucleic acid molecules, which encode novel MCP proteins from *Corynebacterium glutamicum* are described. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing MCP nucleic acid molecules, and host cells into which the expression vectors have been introduced. The invention still further provides isolated MCP 10 proteins, mutated MCP proteins, fusion proteins, antigenic peptides and methods for the improvement of production of a desired compound from *C. glutamicum* based on genetic engineering of MCP genes in this organism.

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